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Effects of elevated carbon dioxide concentration and light on biomass morphology and physiology of black spruce and white spruce seedlings

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EFFECTS OF ELEVATED CARBON DIOXIDE CONCENTRATION AND LIGHT ON BIOMASS, MORPHOLOGY AND PHYSIOLOGY OF BLACK SPRUCE AND WHITE SPRUCE SEEDLINGS

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A CAUTION TO THE READER

This thesis has been through a formal process of review and comment by several committee members. As well, an external examiner has reviewed it.

It is made available for loan by the faculty for the purpose of advancing the practice of professional and scientific forestry.

The reader should realize that any opinions expressed in this thesis are those of the student, and do not necessarily reflect the opinions of the supervisor, the faculty or the University.

ABSTRACT

Jacob Marfo 2008: Effects of elevated carbon dioxide concentration and light on the biomass, morphology and physiology of black spruce and white spruce seedlings Master of Science Forestry thesis, Lakehead University. 119 + xviii pp.

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Carbon dioxide concentration and light interactions influence competition among boreal tree species, though little is known about the exact effects. The interactive effects of [CO_2] and light on growth, biomass production, gas exchange, chlorophyll fluorescence, light response and in-vivo carboxylation of 1-year-old black spruce (Sb) (*Picea mariana* [Mill.] B.S.P) and white spruce (Sw) (*Picea glauca* (Moench Voss) on were investigated. The seedlings were grown under 360 and 720 μmol mol^{-1} [CO_2] at 30, 50 and 100% light in greenhouses for 4.5 months. It was found that root collar diameter (RCD) of Sw decreased with decreasing light while there was no significant difference in Sb between 50 and 30% light. Height was greater at 100% light than shaded. Elevated [CO_2] increased RCD by 33% and enhanced stem-volume by 67, 98 and 84%, respectively at 100, 50 and 30% light. The CO_2 enrichment enhancement of total biomass and net photosynthesis was relatively higher at lower light and greater in Sb than in Sw. CO_2 elevation decreased specific leaf area at 50% light only. CO_2 elevation reduced stomatal conductance (g_s) and transpiration rate (E) and subsequently increased water use efficiency. The reduction in g_s and E increased with decreasing light and much more in Sb than in Sw. CO_2 elevation significantly reduced dark respiration (R_{day}) and the magnitude of reduction was higher in Sw than Sb and with decreasing light. Light compensation point decreased with decreasing

light and that of Sb was much lower at elevated [CO_2]. CO_2 elevation increased light-saturated electron transport rate (J_{max}) and apparent rate of electron transport (J_t) and the increase was highest at 100% light. After 2.5 months, J_{max} was significantly higher in Sb than in white spruce. CO_2 elevation reduced light compensation point and increased the light saturation point. These data suggest that in future climates when atmospheric [CO_2] rises, species competitiveness under low light conditions will increase and the increase will be greater in black spruce than white spruce.

Keywords: boreal forest, gas exchange, growth, biomass allocation, photosynthesis, transpiration, water-use-efficiency, stomatal conductance

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LIST OF ABBREVIATIONS

RCD = rood collar diameter

FOLS = first order lateral shoot

SLA = specific leaf area

SMR = shoot mass ratio

RMR = root mass ratio

SRR = shoot root ratio

Φ_{PSII} = the actual photochemical efficiency of photosystem II

PPFD = Photosynthetic photon flux density

PAR = photosynthetic active radiation

P_n = rate of net photosynthesis

CE = carboxylation efficiency

R_{day} = dark respiration rate during photosynthesis

TPU = triphosphate utilisation

PSII = photosystem II

G_s = stomatal conductance

E = transpiration rate

IWUE = instantaneous water use efficiency

Vc_{max} = maximum carboxylation rate of Rubisco

J_{max} = light-saturated electron transport rate

J_t = apparent rate of electron transport

J_c = partitioning of total electron flow to Rubisco carboxylation

J_o = partitioning of electron flow to Rubisco oxygenation

LCP = photosynthetic light compensation point

LSP = photosynthetic light saturation point

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Chapter 1

INTRODUCTION

The phenomenal rise in atmospheric [CO_2] is one of the most documented global environmental changes in the past 50 years (Prentice *et al.* 2001). The annual rate of increase is approximately 1.9 μmol mol^{-1} (IPCC 2007). Based on complex climatic data available and environmental scenarios, it is modelled that the atmospheric [CO_2] can reach 650–700 μmol mol^{-1} by 2075 and almost 970 μmol mol^{-1} by 2100 (Prentice *et al.* 2001).

Curtis (1996), Tissue *et al.* (1997), Wuebbles *et al.* (1999) Lindner (2000) observed that elevated [CO_2] enhanced photosynthesis and plant biomass production. Again, [CO_2] elevation is known to have significant effects on resource allocation patterns (Larigauderie *et al.* 1988, Huang *et al.* 2007), specific leaf area (SLA) (Zhang *et al.* 2006, Huang *et al.* 2007, Cao *et al.* 2008), branching patterns (Bazzaz 1990, Tissue *et al.* 1997) and stem elongation (Ainsworth and Long 2005). Despite the numerous studies on the effects of [CO_2], little is known about its interactions with other environmental factors such as light (Saxe *et al.* 1998) and species with similar genetic traits. The effects of elevated [CO_2] can be modified by light (Saxe *et al.* 1998). For example, while low light intensities limit photosynthesis (Lambers *et al.* 1998, DeLucia and Thomas 2000) and reduce overall carbon gain and plant growth, high light intensities may also stress plants causing damage to the photosynthetic apparatus, especially when other environmental factors are not at optimum (Lambers *et al.* 1998). Agrell *et al.* (2000) for example reported that the effects of [CO_2] were more pronounced in aspen, birch and maple

grown at high light than at low light. According to Awada and Redman (2001), light conditions affect the biochemistry and morphology of leaves and this translates into changes in photosynthetic and respiratory rates that ultimately affect plant growth. It is well known that elevated [CO_2] lowers the light compensation point to allow plants to grow in shade (Körner 2006) and increase maximum quantum efficiency (measure of photosynthetic efficiency expressed as moles of CO_2 molecules reduced or O_2 molecules evolved per mole of photons absorbed at saturated light (Zeinalov and Maslenkova 1999)) at the expense of reduced photorespiration (Saxe *et al.* 1998). By altering the carbon balance of plants, elevated [CO_2] also influences the competitive ability of several species depending on the light conditions (Bazzaz and McConnaughay 1992).

In the context of rising atmospheric [CO_2], black spruce and white spruce are relevant as they occupy large areas of the boreal forest (Margolis and Brand 1990), which stores the largest amount of carbon in relation to other terrestrial ecosystems (Hollingsworth *et al.* 2006). Again, they are major commercial tree species in the boreal regions. For example, during the 1991-1992 fiscal year, 2.9 million cubic metres of black spruce and white spruce were harvested on Crown land the Northeast Region of Ontario, accounting for approximately 50% of the total volume of all species harvested (Archibald and Arnup 1993).

The intensity and duration of light influence these species dry matter accumulation, bud formation and bud burst as well as their morphology (Colombo and Smith 1984, D'Aoust and Hubac 1986, Colombo *et al.* 2003). The interactions of [CO_2] and light can therefore influence the growth, morphology, biomass and physiology of boreal forest species such as black spruce (*Picea mariana* [Mill.] B. S. P) and white spruce (*Picea glauca* (Moench)).

The objective of this study was to examine the interactive effects of [CO_2] and light on the morphological and biomass characteristics and important physiological traits of the boreal black spruce and white spruce seedlings grown under greenhouse conditions. Chapter two focuses on synthesis of literature relevant to the study. Chapters three and four deal with the morphological/growth and physiological responses respectively. Chapter five provides a general discussion of major outcomes and conclusions.

Chapter 2

LITERATURE REVIEW

Rising carbon dioxide concentration [CO_2] in the atmosphere

The rise in atmospheric [CO_2] is one of the most prominent global changes in the past 50 years (Vitousek 1992, Prentice *et al.* 2001). This phenomenal increase in [CO_2] has been of global interest, especially among biological scientists because of the potential impacts of CO_2-induced global warming and other changes on plants and ecosystems (Prentice *et al.* 2001). The rate of increases have been estimated to range from 1 - 2 (Keeling *et al.* 1995) to 1.9 μmol mol^{-1} $year^{-1}$ (IPCC 2007). The atmospheric [CO_2] in this decade has exceeded the natural range over the last 650,000 years (180 to 300 μmol mol^{-1}) as determined from ice cores (Barnola *et al.* 1987). It is modelled that the atmospheric [CO_2] will reach 650–700 μmol mol^{-1} by 2075 and almost 970 μmol mol^{-1} by 2100 (Prentice *et al.* 2001).

The present increase in atmospheric [CO_2] is caused mainly by anthropogenic emissions of CO_2 (Vitousek 1992, Saxe *et al.* 1998, Prentice *et al.* 2001). According to Prentice *et al.* (2001), about three-quarters of these emissions are due to fossil fuel burning. For instance, fossil fuel burning released an average of 5.4 ± 0.3 Pg carbon annually from 1980 to 1989 and this annual amount increased to 6.3 ± 0.4 Pg between 1990 and 1999. Land use change is responsible for the rest of the emissions (Vitousek 1992, Lambers *et al.* 1998, Prentice *et al.* 2001, Keeling and Whorf 2005). Changes in land use patterns such as tropical deforestation account for the release of 1.6 ± 1.0 Pg of carbon annually (Dixon *et al.* 1994).

The Boreal Forest

The boreal forest is a mixture of coniferous and deciduous tree species. It covers 14.7 million km^2, or 11% of the earth's terrestrial surface (Bonan and Shugart, 1989). The boreal forest is the second largest terrestrial ecosystem, with black and white spruce forests as major components (Hollingsworth *et al.* 2006). It is generally characterized by a mosaic of small to very large areas dominated by 15 conifer and hardwood species from wetlands and uplands (Stewart *et al.* 1998). In comparison to tropical and temperate forests, the net primary productivity of the boreal forest is low (Stewart *et al.* 1998) with an annual production of 1-8 ton dry matter (Melillo *et al.* 1993).

The climate of the Canadian boreal forest is characterized by strong seasonal variation with short, moderately warm and moist summers and long, extremely cold and dry winters (Larson 1980). According to Rumney (1968), the mean annual rainfall of the Canadian boreal forest is relatively low, ranging from 180 to 38 cm in the northwest, 380-510 mm in central Canada to 510-890 mm in eastern Canada, while the European boreal forests have relatively higher rainfall and wetter winters.

Trees in the boreal environment grow under light conditions independent of the prevailing climate, with maximum day length varying from 16 hours at the southern edge of the boreal forest to 24 hours at the northern treeline (Bonan and Shugart 1989). The long-day photoperiod in the region influences the annual cycle of some boreal forest tree species (Koski and Selkainaho 1982). This ensures that growth is initiated and completed when the frost hazard is lowest. The low sun angle in the region is also known to affect forest structure and productivity

through its effect on photosynthesis (Bonan and Shugart 1989). Overall, the region's forest is characterised by short growing seasons and high rates of loss due to fire, pests and disease (van Koorten 1995).

There are large pools of carbon and nitrogen in the soils of boreal forests (van Breemen *et al.* 1998) and the trees store between 40 and 50% of worldwide terrestrial carbon (Schlesinger 1997, Saxe *et al.* 1998). This coupled with the region's low soil temperature (Melillo *et al.* 1993) means that the boreal forest is likely to be the greatest sink for increasing atmospheric [CO_2]. Two things might account for the large carbon contained in the boreal forest; the large size of the area and the edaphic conditions in the region.

Stewart *et al.* (1998) reported that with increasing [CO_2] and temperature, the boreal forest will shift to the current tundra zone or even convert to temperate forest or grassland. On the assumption that soil and moisture conditions would be favourable for forest growth, Rizzo and Wiken (1992) state that climate change in Canada will result in suitable conditions for northward expansion of the boreal forest. Rizzo and Wiken's prediction may not however be a reality since the rate of species migration might be slower than the rate of climate change. Again, the increase in atmospheric [CO_2] is expected to parallel temperature change. If this occurs, then soil moisture might evaporate faster than they are replenished in some areas of the boreal forest. Overall, climate change is expected to decrease the size of the boreal forest and its biomass and carbon stock and increase the proportion of younger stands within it (Stewart *et al.* 1998).

Silvics of black spruce and white spruce

Black spruce and white spruce are major commercial tree species in the boreal regions and are planted over larges areas across Canada (Wang and Su 2002). For instance, out of the 1,264,964 hectares of forest plantation established in Canada between 1980 and 1985, white spruce and black spruce represented 37 and 21% respectively of this area (Margolis and Brand 1990). In the boreal forest, black spruce is one of the most desirable tree species because of its superior fibre quality (Inderjit and Mallik 1996) and is regarded as the most abundant and most economically important boreal species in North America (Bigras and Bertrand 2006). For example, during the 1991-1992 fiscal year, 2.9 million cubic metres of black spruce and white spruce were harvested on Crown land the Northeast Region of Ontario, accounting for approximately 50% of the total volume of all species harvested in the region (Archibald and Arnup 1993).

According to Viereck and Johnston (1990), black spruce has a broad range of habitat spanning from Northern Massachusetts in the United States to Northern Labrador in Canada to the west coast of Alaska. The species has wide ecological amplitude and grows on sites ranging from dry sands, gravels, and shallow soils over bedrock, through deep nutrient-rich mineral soils on uplands to waterlogged, nutrient-deficient sphagnum peats on lowlands (Sims *et al.* 1990, Haavisto and Jeglum 1995).

The average annual height growth of black spruce seedlings varies from 2.5 to 15 cm when grown on mineral substrates (Viereck and Johnston 1990), with mature trees reaching a height of 7 to 30 m, depending on site conditions (Haavisto and Jeglum 1995). The branching of black

spruce occurs in the early stages of development (Bégin and Filion 1999). Black spruce has a plate-like shallow root system with numerous adventitious roots which readily develop in humid peatland conditions (Haavisto and Jeglum 1995) and no tap root (Viereck and Johnston 1990). In a few cases however, the roots may penetrate to 60 cm deep, though the bulk of the root biomass still remains in the top 20 cm of the growing medium (Viereck and Johnston 1990). Because black spruce roots are mainly in the top few centimetres of the soil, hence, their photosynthetic rate can be sustained over longer period with little or no pot-induced downregulation in relation to white spruce

Black spruce forests occur in different environmental conditions, which are very important in the context of climate change (Hollingsworth *et al.* 2006). This is because of the large amount of carbon stored in the soils black spruce grow and the frequent fire disturbance (Vogel *et al.* 2005). Although the species occurs in different edaphic and climatic regimes, it is found mainly in muskegs with poor drainage and low nutrients (Vincent 1965, Larson 1980, Patterson *et al.* 1997). Black spruce growing in muskegs has a high metabolic efficiency in the use of nutrients to produce new biomass, a high degree of drought tolerance, a high efficiency in its utilisation of water and a greater biomass allocation to roots (Patterson *et al.* 1997). According to Patterson *et al.* (1997), black spruce is also able to tolerate nutrient stress better than white spruce.

According to Viereck and Johnston (1990), the soil moisture regimes of black spruce sites typically range from humid to sub-humid. Minimum and maximum temperatures for black spruce growth range from -11°C to 27°C and in extreme areas from -62°C to 41°C (Viereck and

Johnston 1990). The total annual precipitation at black spruce sites ranges from 380 mm to 760 mm.

Black spruce is an intermediate shade tolerant species (Baker 1949, Ritchie 1959, Nienstaedt and Zasada 1990, Haavisto and Jeglum 1995) and its photosynthetic rate usually reaches saturation at 25–50% of full sunlight (Grossnickle 2000). Vincent (1965) indicated that reducing light levels to 25% would not reduce the height growth young trees substantially, but maximum growth is achieved at full light. Light intensity influences dry matter accumulation (D'Aoust and Hubac 1986) while bud formation and freezing tolerance of black spruce seedlings is highly sensitive to photoperiod (Colombo and Smith 1984). Short day length is known to shorten black spruce terminal bud terminal buds initiation and reduce seedling root collar diameter and shoot dry weight. Colombo *et al.* (2003)

Black spruce may form pure stands or grow in association with white spruce, balsam fir (*Abies balsamea*), jack pine (*Pinus banksiana*) and quaking aspen (*Populus tremuloides*), as well as paper birch (*Betula papyrifera*), lodgepole pine *(Pinus contorta),* balsam poplar, northern white-cedar (*Thuja occidentalis*), black ash (*Fraxinus nigra*), American elm (*Ulmus americana*), and red maple (*Acer rubrum*) (Viereck and Johnston 1990).

The North American boreal white spruce has a transcontinental range from Newfoundland and Labrador west across Canada, almost reaching the Arctic Ocean at latitude 69° N (Nienstaedt and Zasada 1990). The species is adapted to a wide range of edaphic and climatic conditions and grows from sea level to 1520 m elevation (Nienstaedt and Zasada 1990).

White spruce grows on a wide variety of soils of glacial, lacustrine, marine, or alluvial origin (Nienstaedt and Zasada 1990) generally on drier, nutrient rich upland sites (Vincent 1965, Larson 1980). The rich nature of soils the species grows on is likely due to the high nutrient content of the parent material. For example, soils of alluvial origin (river sediment deposits) are usually nutrient-rich. According to Nienstaedt and Zasada (1990), the species has greater demand for nutrient, moisture, light and other growth resources than associated conifers such as black spruce, making the range of land supporting its growth more limited with increasing climate severity. The species is considered "plastic" (Nienstaedt and Zasada 1990) because of its ability to repopulate areas at the end of the glacial period and can grow under extreme climate and soil conditions. White spruce usually grows in association with jack pine, balsam fir, black spruce, aspen and paper birch in the boreal forest.

Annual minimum and maximum rainfall reported in the species range is 250 and 1270 mm respectively, with mean annual figures of 380 and 510 mm reported in Alberta, Saskatchewan, and Manitoba. The mean annual rainfall reported here for white spruce far exceeds that within the range of black spruce. This is because the soils black spruce grows on have high water retention capacity (muskegs to alluvial origin soils) than that found for white spruce. Temperatures ranging from as low as -54°C to as high as 43°C have been reported in some range of the species, though the mean winter and summer temperatures are -29°C and 24°C (Nienstaedt and Zasada 1990).

Alternating day/night temperatures of 25°/20°C are considered the best for the growth of white spruce seedlings (Tinus *et al.* 1979, Nienstaedt and Zasada 1990). According to Nienstaedt and

Zasada (1990), the photoperiod for successful growth of white spruce varies from 17-24 hours with photoperiod below 14 hours resulting in growth cessation in seedlings. Long photoperiods with photosynthetically active radiation above 244 μmol m^{-2} s^{-1} result in increased dry matter production while short photoperiods induce dormancy for the formation of needle primordia (Nienstaedt and Zasada 1990).

Like black spruce, the growth of white spruce seedlings is greatest at full light intensity (Nienstaedt and Zasada 1990) with maximum photosynthesis occurring at 40–60% of full sunlight (Man and Lieffers 1997) and maximum diameter and stem volume at full light (Lieffers and Stadt 1994). According to Nienstaedt and Zasada (1990), growth at 50% light result in 25% height reduction, 50% shoot mass reduction and 40% less root penetration. The minimum light required for the survival of white spruce seedlings range from 8% (Lieffers and Stadt 1994) to 15% (Chen 1997) of full sunlight. Logan (1969) reported 11–13% of sunlight as the minimum for the survival of white spruce seedlings while Eis (1970) reported the death of white spruce seedlings below 15% light.

Increasing [CO_2] and forest ecosystems

The potential effects of increasing [CO_2] on forests and other natural ecosystems generated interest only in the later part of the twentieth century, although elevated [CO_2] stimulation had been long used in plant growth, especially in horticulture (Nowak *et al.* 2004). The growing interest in the impacts of elevated atmospheric [CO_2] on forest trees and forest ecosystems is mainly because forests cover about 43% of the earth's surface (Melillo *et al.* 1993) and account for about 70% of terrestrial net primary production (Melillo *et al.* 1993, Lal 2005).

Numerous studies (D'Arrigo *et al.* 1987, Bonan and Shugart 1989, Lambers *et al.* 1998, IPCC 2007) have predicted that the north-temperate and boreal forests are likely to be the greatest sink for the rise in atmospheric [CO_2] because of their location the large fossil material accumulated in the region. The boreal forest previously as carbon neutral is now known to be net sink (Dixon *et al.* 1994) and they store significantly more carbon in their soils and associated peatlands than do tropical forests (van Kooten 1995). This makes the boreal biome much more important as a global carbon sink (Kronberg and Fyfe 1992). Within the boreal zone, species from forested biomes may respond to elevated [CO_2] more than species from grassland biomes, providing evidence that forests are acting as major sinks for increasing levels of atmospheric [CO_2] (Saxe *et al.* 1998, Körner 2006).

Contrary to the many findings that the boreal forest would be impacted greatly by rising atmospheric [CO_2], in a free-air enrichment carbon dioxide experiment (FACE) in the Mojave Desert, Smith *et al.* (2000) reported that arid ecosystems such as desert, which occupy about 20% of the earth's terrestrial surface area, would be the most responsive ecosystem type to elevated atmospheric [CO_2] and associated global climate change. In contrast, Llord and Farquhar (1996) reported that low altitude tropical forests are the major carbon sink, hence to be affected most by rising [CO_2]. Little evidence however supports the claims that the arid ecosystems and tropical forests would be affected most by the rising atmospheric [CO_2]. The location of these ecosystems does not make them more susceptible to the rising atmospheric [CO_2] in relation to the boreal forest. Though tropical deforestation contributes to the rising [CO_2], the effects and impacts are less than in the boreal forest.

Within the same ecosystem, it has been predicted that C_3 plants will give higher response to elevated [CO_2] than their C_4 counterparts (Curtis and Wang 1998). The rate of net [CO_2] assimilation is not saturated in C_3 plants at 350 μmol mol^{-1}, hence the rise in [CO_2] is more likely to enhance photosynthesis in C_3 plants than in C_4, where the net assimilation is virtually saturated at this (Bowes 1993, Lloyd and Farquhar 1994, Körner 2006). According to Körner (2006), plants with shorter life span and determinate growth and plants that grow in closed canopies benefit less from increased [CO_2]. Comparisons between trees and shrubs and non-woody groups did not produce significant differences (Nowak *et al* 2004).

According to Bolker *et al.* (1995), plant communities with greater biodiversity would be more responsive to elevated [CO_2] than where plant diversity is less. This is however very debatable since competition for all resources may increase with increased diversity reducing the CO_2 impact (Karnosky 2003). Contrary to Bolker *et al.* (1995) findings, Dukes and Mooney (1999) reported that many plants responded positively to elevated [CO_2] when they were grown individually or in monoculture and their response to increased [CO_2] may be down regulated when grown in mixed plant communities. For example, plant species such *Chenopodium album* that are known to respond positively to elevated [CO_2] in monoculture responded less predictably to CO_2 enrichment when they are grown in association with other species.. Dukes (2002) using the highly invasive plant species, *Centaurea solstitialis* (yellow star thistle) again reported that plants grown in monoculture responded strongly to [CO_2, increasing aboveground biomass production by 70%, inflorescence production by 74%, and midday photosynthesis by an average of 132% than those grown within a mixed-species environment. Dukes (2002) findings seem more plausible in the context of increasing [CO_2]. Competition for resources

such as moisture, light, nutrients increase in most cases when different species are grown together. Bolker *et al.* (1995) results may be valid when each of the multi-species have different requirements and tend to be symbiotic in nature.

Effects of elevated [CO_2] and photosynthesis

The present atmospheric [CO_2] is not enough to saturate Ribulose-1, 5-bisphosphate carboxylase-oxygenase (Rubisco) C_3 plants (Curtis and Wang 1998, Körner 2006) and is therefore a limiting factor to photosynthesis, growth and productivity of many plants (Vu *et al.* 1997, Huang *et al.* 2007). The photosynthetic rate within the leaf results from the direct activity of Rubisco (Vu *et al.* 1997, Lambers *et al.* 1998, Huang *et al.* 2007). The activity of Rubisco in the leaf is greatly influenced by the [CO_2], temperature and light intensity (Vu *et al.* 1997). Simultaneous to carbon fixation, the competing process of oxygenation also catalysed by Rubisco occurs and the net rate of photosynthesis per unit leaf area of C_3 plants is affected by the ratio of [CO_2] to [O_2] since both processes compete at the active site of Rubisco for the primary acceptor Ribulose-1,5-bisphosphate (RuBP) (Körner 2006, Huang *et al.* 2007). Elevated atmospheric [CO_2] however is able to increase the intercellular [CO_2] and the CO_2:O_2 ratio at the site of carboxylation to the disadvantage of oxygenation, since both processes are catalysed by the same enzyme (Saxe *et al.* 1998, Korner 2006, Huang *et al.* 2007).

By inhibiting respiration and favouring photosynthesis, elevated [CO_2] generally enhances net photosynthesis of plants. Enhanced net photosynthetic rate due to CO_2 elevation have been reported by Drake *et al.* (1997) Curtis and Wang (1998), Zhang and Dang (2005), Körner 2006, Huang (2007) For example, Saxe *et al.* (1998) reported 67% net photosynthesis enhancement in field grown *Pinus taeda* L. exposed to elevated [CO_2]. According to Ainsworth and Long

(2005), plants exposed to elevated [CO_2] in FACE experiment resulted in light-saturated leaf photosynthetic rate of 31% and 21% increase in diurnal photosynthetic carbon assimilation.

The increase in photosynthetic light use efficiency under elevated [CO_2] arises from changes in Rubisco kinetics and light-trapping efficiency (Griffin *et al.* 2001). Because elevated [CO_2] is able to increase both the number of mitochondria and chloroplastic stroma at the carboxylation site (Griffin *et al.* 2001), there is more efficient utilisation of light and the plants are able to fix more carbon.

Photosynthetic down-regulation sometimes occurs when species are exposed to elevated [CO_2] over longer durations (Drake *et al.* 1997, Lambers *et al.* 1998, Ainsworth and Long 2005, Körner 2006, Huang *et al.* 2007). Down-regulation is the process by which a cell decreases the number of cellular components, such as RNA or protein, in response to an external variable. This is usually linked with insufficient sink demand, or reallocation of nitrogen away from the photosynthetic apparatus to meet other demands within the plant (Bowes 1991). It has been suggested (Stitt 1991) that decreased levels of Rubisco and other photosynthetic enzymes may result from an accumulation of carbohydrates, reflecting an imbalance between photosynthate formation and utilisation at elevated [CO_2]. Johnsen (1993) reported photosynthetic down-regulation in black spruce exposed to elevated [CO_2] and attributed the observation to the small size of the pot the seedlings were growing in. In a review by Ceulemans and Mousseau (1994), small pot size was the main (78%) cause of down-regulation. A generalised photosynthetic CO2 response curve is shown in Fig. 2.1

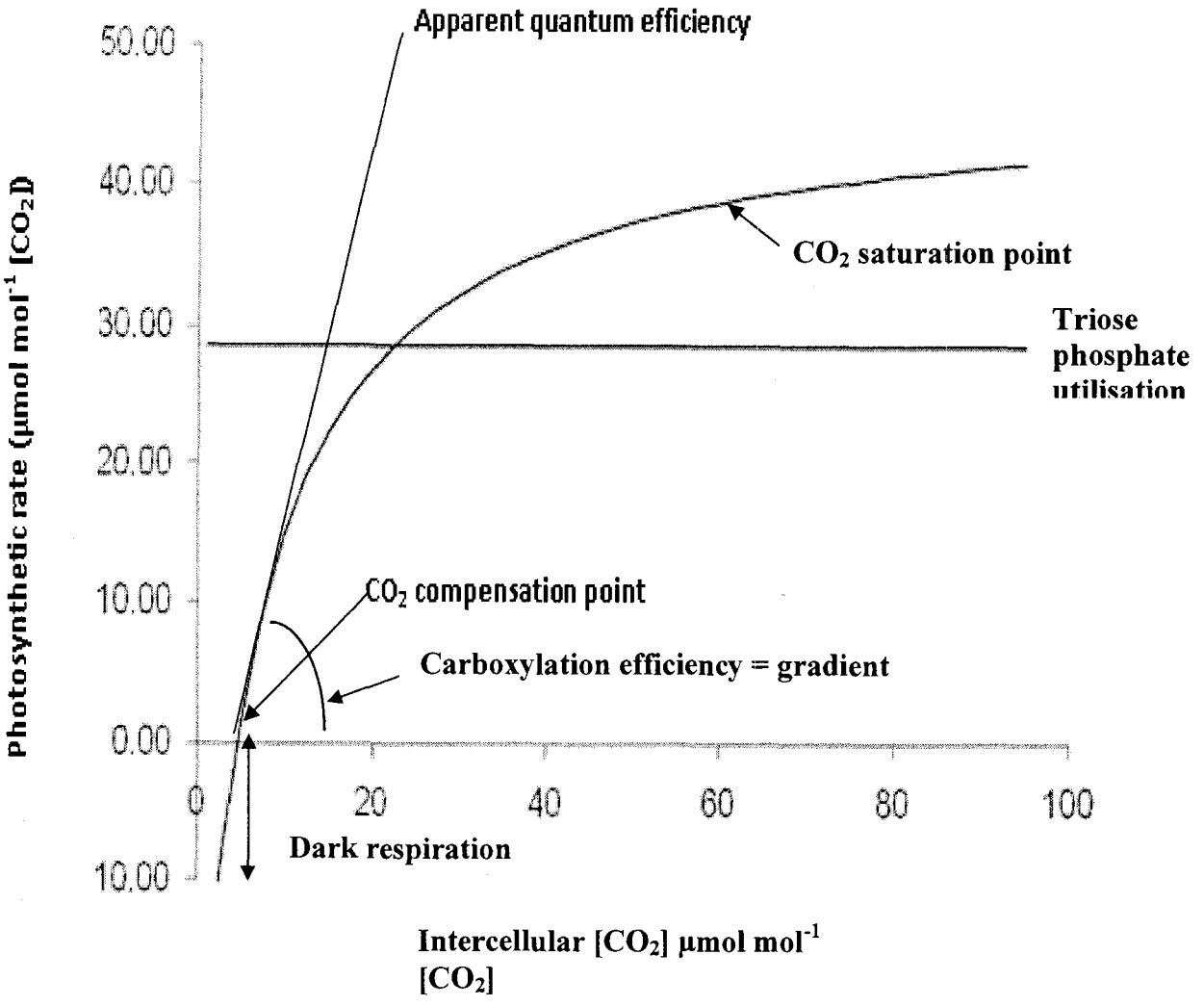

Fig. 2.1: Generalised *A*-C_i curve

Elevated [CO_2] effects on stomatal conductance

The opening and closing of the stomata controls the movement of CO_2 in and out of the leaves (Lambers *et al.* 1998). The rate of passage of water vapour and gases such as carbon dioxide through mostly the stomata defines stomata conductance (g_s). Paoletti and Gellini (1993) suggest that the main factor for reduction in stomatal conductance at elevated [CO_2] is not the reduction in stomatal density or stomatal pore area, but rather the reduction in the stomatal

opening. In the long term however, decrease in g_s is caused by changes in stomatal density or stomatal index (the percentage of epidermal cells that are guard cells) and stomatal aperture (Ainsworth and Rogers 2007)

In relation to [CO_2], the stomata of most species is known to close with increasing [CO_2] (Drake *et al.* 1997) leading to increased water use efficiency (Ainsworth and Long 2005, Körner 2006, Huang *et al.* 2007). However, the exact mechanism by which stomata open or close in response to extracellular [CO_2] remains unclear (Mott 1990), though it is highly linked with malate synthesis which controls the anion channels in the guard cell plasma membrane (Hedrich and Marten 1993). The opening and closing of the stomata is also influenced by air vapour pressure, the transpirational rate of the plant as well as air temperature (Lambers *et al.* 1998).

According to Saxe *et al.* (1998), the stomatal response of trees to elevated [CO_2] is usually less than that of crops and shrubs. Elevated [CO_2] is known to generally cause an approximately 20% reduction in stomatal conductance of trees (Field *et al.* 1995) and 40% reduction in shrubs (Morison 1987). In a meta-analysis of studies lasting at least a year, Medlyn *et al.* (2001) reported a 21% decrease in stomatal conductance of trees under elevated [CO_2], with younger trees having greater reduction and hardwoods being more sensitive than conifers. In another meta-analysis involving 48 studies with woody plants, Curtis and Wang (1998) reported a non-significant 11% reduction in stomatal conductance under elevated [CO_2]. Ainsworth and Long (2005) also reported a similar 20% reduction in stomatal conductance of plants exposed to elevated [CO_2] using FACE. In some studies however, the increased [CO_2] did not cause any

stomatal response. For example, the stomata of *Xanthium strumarium* grown in greenhouse failed to respond to the elevated [CO_2] (Drake *et al.* 1997).

Elevated [CO_2] effects on transpiration and water use efficiency

Stomatal responses to elevated [CO_2] could alter transpiration rate and soil water depletion thereby affecting local climate (Saxe *et al.* 1998). When leaves are exposed to dry air, transpiration rate increases due to the greater vapour pressure deficit between the leaf and the atmosphere (Lambers *et al.* 1998). However, at higher [CO_2], stomatal aperture and conductance are reduced, thereby resulting in a reduced transpiration rate (Drake *et al.* 1997). Lodge *et al.* (2001) exposed *Quertcus myrtyfolia* Willd. to a doubled ambient [CO_2] and reported that the elevated [CO_2] reduced stomatal conductance by 40%, resulting in a corresponding 19% decrease in the transpiration rate. Li *et al.* (2003), working with the same species, also reported that elevated [CO_2] caused a significant reduction in stomatal conductance, resulting in a 45% mean reduction of transpiration rate. Zhang and Dang (2005) recently reported that elevated [CO_2] reduced leaf transpiration rate of jack pine and white birch and caused a subsequent enhancement of water use efficiency. Except for jack pine (*Pinus banksiana*), Tjoelker *et al.* (1998) reported that elevated [CO_2] resulted in a decline in mean stomatal conductance of aspen, paper birch , tamarack, black spruce resulting in a 40 to 80% increase in water use efficiency.

From most of these studies, it is evident that at least at the leaf level, when stomatal conductance is reduced in direct response to elevated [CO_2], transpiration rate is reduced resulting in subsequent increase in water use efficiency.

Effects of elevated [CO_2] on above ground growth and biomass

Growth and above-ground biomass production generally increases with exposure to elevated [CO_2] (Ainsworth and Long 2005), though the response varies with species type (Saxe *et al.* 1998), experimental conditions (Ainsworth and Long 2005, Huang *et al.* 2007) and duration of the experiment (Körner 2006). In hardwoods, elevated [CO_2] is known to increase leaf area index the number of leaves and branches and changes canopy structure (Tissue *et al.* 1997).

In an open top chamber experiment, Tissue *et al.* (1997) reported that elevated [CO_2] increased the leaf area of loblolly pine by 217% in the first growing season and by 80, 58 and 41% in the subsequent three growing seasons. The leaf area of *Populus* clones grown under elevated [CO_2] were 8–18% higher than at ambient [CO_2] (Ceulemans *et al.* 1995). According to Ceulemans and Mousseau (1994), the increase in total leaf area of plants exposed to elevated [CO_2] is due to a larger individual leaf area or to larger numbers of leaves produced and the increase in individual leaf area is associated with an increase in the number of cells per leaf (Gaudillere and Mousseau 1989) or a greater rate of leaf cell expansion through changes of cell wall properties (Taylor *et al.* 1993). Ranasinghe and Taylor (1996) reported that the increased leaf area under elevated [CO_2] resulted from increased leaf cell expansion independent of leaf cell production. In 12 species grown under field conditions, Ainsworth and Long (2005) reported that elevated [CO_2] increased leaf area by 8% over ambient [CO_2]. They further reported that trees had a 21% (higher) leaf area index over shrubs to allow for rapid canopy closure.

In some studies however, elevated [CO_2] failed to increase leaf area. For example, Norby *et al.* (1992) reported that in *Liriodendron tulipifera*, total leaf area was reduced under elevated [CO_2] and minimized the overall effects of elevated [CO_2] on growth rates suggesting that this was due to a reduction of the individual leaf area and not due to decreased leaf production rate.

Though in some few cases total leaf area of plants were either not affected or reduced by elevated [CO_2], it can be concluded that leaf area is generally increased under elevated [CO_2] and the magnitude on increase depends on the plant functional group.

White total leaf area is increased under elevated [CO_2] specific leaf area (ratio of leaf area per unit dry mass) is known to reduce under elevated [CO_2] (Wolfe *et al.* 1998, Zebian and Reekie 1998, Zhang *et al.* 2006). Zhang *et al.* (2006) reported a reduction in specific leaf area in white beech seedlings grown at elevated [CO_2]. In a review of studies under field conditions, Ainsworth and Long (2005) reported that elevated [CO_2] decreased SLA by 6%. In some cases however, SLA failed to respond to increased [CO_2]. For example, Springer and Thomas (2007) reported that elevated [CO_2] did not have any effect on the SLA of *A. rubrum, C. glabra, C. canadensis or L. tulippefera.*

In general, the reduction in SLA in plants exposed to elevated [CO_2] is due to changes in leaf structure, anatomy and/or accumulation of soluble carbohydrates (Lambers *et al.* 1998, Zhang *et al.* 2006). It seems that the reduction in SLA under elevated [CO_2] is not due to solely changes in the structure and anatomy of leaves. Since SLA is a ratio, a higher per unit increase in biomass to leaf area results in reduced SLA under [CO_2] elevation.

Numerous studies account for increases in plant height and root collar diameter as well as the number of branches in plants grown in enriched [CO_2] (Gunderson and Wullschleger 1994, Tissue *et al.* 1997). For example, Tissue *et al.* (1997) reported that elevated [CO_2] significantly increased the number of branches of loblolly pine which resulted in changes in crown size and structure. Ainsworth and Long (2005) in a meta-analysis also reported that elevated [CO_2]

increased plant height and diameter with trees showing greater response over shrubs and that plant height increased by 14% under elevated [CO_2] during third year of exposure. They further reported that stem diameter was increased by 5% while number of branches had a 25% increase over ambient [CO_2] under field conditions. Johnsen and Major (1998) reported that seedlings grown under elevated [CO_2] had a 20% greater height, 16% greater RCD, 78% more branches, 20% longer average branch length, and a 94% greater cumulative branch length. Zhang and Dang (2007) also reported an average of 11% increase in height in white birch, white spruce, and black spruce grown in elevated [CO_2]. There have been a few cases, however, where elevated [CO_2] failed to stimulate increase height growth. For instance, Zhang *et al.* (2006) reported that elevated [CO_2] failed to enhance height and diameter of birch seedlings grown under greenhouse conditions.

The growth of trees is directly related to the light intercepted by the foliage which in turn depends on the leaf area and canopy structure as well the height and diameter and number of branches (Saxe *et al.* 1999, Ward and Strain 1999, Huang *et al.* 2007). Because elevated [CO_2] stimulates these variables, it is able to change canopy structure and improve growth and plants' competitive ability (Saxe *et al.* 1998). The greater stem diameter and height and subsequent greater stem volume associated with elevated [CO_2] results in greater hydraulic conductivity in trees (Saxe *et al.* 1998).

The number of studies reporting above ground biomass enhancement under elevated [CO_2] is large (Drake *et al.* 1997, Lambers *et al.* 1998, Saxe *et al.* 1998, Bigras and Bertrand 2006, Huang *et al.* 2007). Zhang and Dang (2007) reported an enhancement of above ground biomass

production in black spruce, white spruce, jack pine and trembling aspen grown under elevated [CO_2]. In a recent meta-analysis of tree species, Wang (2007) reported that elevated [CO_2] increased above ground biomass by 31% at the population level and 23% at the community level. Norby *et al* (2005) also observed a 23% enrichment in the above ground biomass of forest species under field conditions. According to Saxe *et al.* (1998), conifers increased total biomass by 130% after a mean exposure of 338 days while hardwoods had a 49% biomass enhancement after 329 days of exposure to elevated [CO_2]. According to Ceulemans and Mousseau (1994), the biomass increase result from an extra amount of assimilates which are partitioned into different plant structures, leading to distinctive root/shoot balances.

Below ground growth and biomass under elevated [CO_2]

Root growth under elevated [CO_2] consists of root length extension to permit exploration of broader soil zones, branching to allow more thorough soil patch exploration and secondary growth which allows more effective transport and storage (Kubiske and Godbold 2001). Elevated [CO_2] is known to enhance below ground growth and biomass production in plants (Zak *et al.* 1993, Bernston and Bazzaz 1996, Day *et al.* 1996, Saxe *et al.* 1998, Zhang *et al.* 2006). Bernston and Bazzaz (1996) reported that elevated [CO_2] increased total root growth and biomass of white birch seedlings though it failed to increase the net growth and biomass of red maple. Zhang *et al.* (2006) recently reported that elevated [CO_2] increased root biomass of white birch seedlings with no effect on the root mass ratio (ratio of root mass to total mass). Zak *et al.* (1993) observed a significant increase in the number of roots, root length and shoot-to-root growth in *Populus grandidentata* Michx. In an oak-palmetto scrub ecosystem in central Florida, Day *et al.* (1996) observed a significantly higher growth in fine roots in elevated

[CO_2]. Under a field situation, Norby *et al.* (2004) also reported that fine root growth increased by 100% under elevated [CO_2]. Lukac *et al.* (2003) reported a significantly greater biomass allocation in three *Populus* species and root biomass enhanced by 47-76% under elevated [CO_2]. Kubiske and Godbold reported that root growth under elevated [CO_2] was increased by a factor of 1.3 over that of plants grown at ambient [CO_2].

The response of plant roots to [CO_2] depends on the experimental conditions (Ceulemans and Mousseau 1994), especially the nutrient regime (Zhang and Dang 2007) and water availability (Saxe *et al.* 1998). The flow of carbon through the roots into the soil is one of the key processes in understanding the functioning of a forest ecosystem under elevated CO_2 (Ceulemans and Mousseau (1994). Thus, the general increase in root biomass, root length and volume under elevated [CO_2] is likely to enhance carbon flow. The root response to [CO_2] is of critical importance in the storage of carbon in forest ecosystems which subsequently affect water and nutrient acquisition (Saxe *et al.* 1998)

Photochemical quantum yield of Photosystem II under elevated [CO_2]

The quantum yield of PSII is the effective quantum yield of PSII (Φ_{PSII}) calculated from the oxidised fraction of PSII and the efficiency of energy transfer from light-harvesting complexes of photosystem II to the reaction centre (Genty *et al.* 1989). PSII ((Fḿ -F)/Fḿ or ΔF/ Fḿ)) is the efficiency of PSII under the actual degree of reaction centre closure (Rosenqvist and van Kooten 2003). The Φ_{PSII} is related to the electron transport rate (ETR) in the leaf as

$$ETR = \Phi_{PSII} * PPFD * 0.5 * 0.84$$

Where PPFD is the incident photon flux density, 0.84 is the fraction of incident light that is absorbed by the leaf and the 0.5 is the approximate fraction of light directed to the PSII (Genty *et al.* 1989).

The Φ_{PSII} response to $[CO_2]$ is of a diverse magnitude, ranging from no effect (Pospisilova *et al.* 1999, Zhang and Dang 2005) to an increased (DeLucia and Thomas 2000) to reduction Φ_{PSII} (Zhang and Dang 2005). DeLucia and Thomas (2000) studied photosynthetic responses of *A. rubrum, C. glabra, C. canadensis* and *L. styraciflua* to CO_2 enrichment and reported that with the exception of *A. rubrum*, elevated $[CO_2]$ increased the Φ_{PSII} response of the remaining species.

Cheng *et al.* (2001) observed that Φ_{PSII} responded linearly as photosynthetic assimilation in response to intercellular $[CO_2]$ though it plateaued earlier and since assimilation rate increases with increasing internal $[CO_2]$, it can be concluded that Φ_{PSII} increased with increasing $[CO_2]$. In mature loblolly pines, Hymus *et al.* (1999) observed that Φ_{PSII} was higher under elevated $[CO_2]$ and they attributed the increase to changes in photochemical quenching coefficient. Scarascia-Mugnozza and colleagues (1996) reported that with optimal soil moisture, Φ_{PSII} was increased by 93% in oak grown for 3 years at elevated $[CO_2]$.

Pospisilova *et al.* (1999) reported that elevated $[CO_2]$ had no effect on tobacco grown under CO_2 enrichment. In a recent study of 11 species, Wang *et al.* (2008) reported that elevated $[CO_2]$ failed to stimulate Φ_{PSII} changes except in tomato and wheat which had a higher Φ_{PSII} under elevated $[CO_2]$. Elevated $[CO_2]$ increased Φ_{PSII} by 20-70% in one poplar clone and reduced it by 10-30% in another (Besford *et al.* 1996). Wang (1996) also documented that the

interaction between elevated temperature and [CO_2] increased Φ_{PSII} whereas elevated [CO_2] by itself caused a reduction in the Φ_{PSII} of 1-year old needles in Scots pine trees exposed to elevated [CO_2] for 4 years. Zhang and Dang (2005) reported that elevated [CO_2] decreased Φ_{PSII} after 2.5 months though there was no effect after 4.5 months.

The effects of [CO_2] on chlorophyll photochemical processes are difficult to interpret because they are not necessarily rate-limiting processes in regulating tree growth responses to [CO_2], but are genotype-dependent and environment-dependent (Drake *et al.* 1997). According to Drake and colleagues (1997), it is most likely that the photochemical reactions respond to elevated [CO_2] by a combination of feedback regulations from substrate regeneration, Rubisco activity, and photosynthate accumulation and export.

Elevated [CO_2] effects as modified by light

Light is the most important abiotic factor affecting plant establishment, growth and survival (Poorter 2001). The photosynthetically active radiation-PAR (400 – 700 nm) directly drives photosynthesis (Lambers *et al.* 1998). Poorter (2001) further states that plant performance is enhanced through morphological and physiological acclimation to the light environment. Light intensity affects the biochemistry and morphology of leaves and this translate into changes in photosynthetic and respiratory rates to affect plant growth (Awada and Redman 2001). Higher levels of carbon dioxide have a positive effect on plant growth in both low and high light Zeebian and Reekie (1998). For example, Agrell *et al.* (2000) reported that the effects of elevated [CO_2] were more pronounced in aspen, birch and maple grown at high light. Despite

the key role light plays in the modification of CO_2 responses, little work has been done on elevated [CO_2] and light interactions (Saxe *et al.* 1998).

While low light intensities may pose a threat to plants survival by limiting photosynthesis (DeLucia and Thomas 2000) and reducing net carbon gain hence plant growth. High light intensities on the other hand stress plants causing damage to the photosynthetic apparatus, especially when other factors are not at optimum (Lambers *et al.* 1998). However, the strong positive interaction between light level and CO_2 enrichment always serve to minimise this effect (DeLucia and Thomas 2000).

Elevated [CO_2] lowers the light compensation points to allow plants to grow in shade (Körner 2006) and increases maximum quantum efficiency by reducing photorespiration (Saxe *et al.* 1998). Responses of trees grown at low light levels show that photosynthesis is almost always increased by elevated [CO_2] with the degree of increase dependant on other abiotic factors such as nutrient levels.

Based on relative growth enhancement, Hättenschwiler and Körner (2000) reported that interaction between light and [CO_2] resulted in noticeable changes in species shade tolerance and growth rate ranking. For example, in an experiment conducted in the understorey of a tropical forest, one woody species experienced growth stimulation by elevated [CO_2] only at very low light levels whereas the CO_2 response of the other four species did not depend on light environment (Wurth *et al.* 1998). Elevated [CO_2] also influence the competitive ability of several species by altering their carbon balance and this depends on the light conditions

(Bazzaz and McConnaughay 1992). The interactions of light and [CO_2] therefore have important implications on the growth, morphology, biomass and physiology of black spruce and white spruce.

The few studies on the interactive effects of [CO_2] and light provide conflicting results on the exact direction of the effects. Again, most of these studies focus just on morphological aspect and in most cases studies are done on crops rather than on forest tree species. For example while some authors (Johnsen and Major 1998, Herrick and Thomas 1999, Walters and Reich 2000), indicate that the effects of increased [CO_2] is masked at low light intensities, hence limited photosynthesis, others (Teskey and Shrestha 1985, Saxe *et al.* 1998, Körner 2006) report that because respiration rates are reduced at lower light intensities, the relative effect of increased [CO_2] is higher. The strong positive interaction between light level and CO_2 enrichment serve to ensure photosynthesis is still high at low light intensities under elevated [CO_2] (DeLucia and Thomas 2000). It is therefore hypothesized that elevated [CO_2] will enhance the growth, biomass and photosynthetic rate of both species. Shade tolerant plants are better able to take advantage of increase [CO_2] than relatively less shade tolerant species (Teskey and Shrestha 1985). As such, it is expected that the growth, biomass and photosynthetic enhancement of black spruce will be higher than that of the less shade tolerant white spruce.

Chapter 3

Interactive Effects of Carbon Dioxide Concentration and Light on the Morphological and Biomass Characteristics of Black Spruce and White Spruce Seedlings[1]

Abstract

CO_2-light interactions can influence the competition among boreal plants, but are poorly understood. Such interactions on growth and biomass of 1-year-old black spruce (Sb) (*Picea mariana* [Mill.] B.S.P) and white spruce (Sw) (*Picea glauca* (Moench) Voss) under 360 and 720 μmol mol^{-1} [CO_2] and 30, 50 and 100% light in greenhouses was investigated. There were significant 2-way and 3-way interactions. Root collar diameter (RCD) of Sw decreased with decreasing light while there was no significant difference in Sb between 50 and 30% light. Height was greater at 100% light than shaded. Elevated [CO_2] increased RCD by 33%. It enhanced stem-volume by 67, 98 and 84%, respectively at 100, 50 and 30% light. The CO_2 enhancement of total biomass was relatively higher at lower light and greater in Sb than in Sw. CO_2 elevation decreased specific leaf area at 50% light only. Root mass was generally higher at 100% light than shaded. [CO_2] elevation increased the root mass of Sb at 100% light but decreased it at 30% light. CO_2 elevation decreased shoot/root ratio at 100% light but increased it at 30% light. Our data suggest that CO_2 elevations will likely increase species competitiveness under low light conditions and the increase will be greater in shade tolerant than less tolerant species.

Keywords: Boreal forests, elevated [CO_2], [CO_2]-light-species interactions, black spruce, white spruce seedlings, climate change

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Introduction

The global atmospheric carbon dioxide concentration [CO_2] has increased from the pre-industrial value of 280 μmol mol^{-1} to approximately 379 μmol mol^{-1} in recent years, and the present annual [CO_2] increase rate of 1.9 μmol mol^{-1} is the highest on record (IPCC 2007). Research has shown that [CO_2] elevation has great impact on plant growth and biomass production (Curtis and Wang 1998; Ward and Strain 1999). Numerous studies (Curtis 1996; Tissue *et al.* 1997, Wuebbles *et al.* 1999, Lindner 2000) showed that elevated atmospheric [CO_2] enhance photosynthesis and plant biomass production. Ceulemans and Mousseau (1994) found that a doubling of atmospheric [CO_2] increases leaf level photosynthesis by approximately 40% in conifers and 60% in deciduous trees. Zhang and Dang (2007) reported an enhancement of biomass production in black spruce (*Picea mariana* [Mill.] B.S.P), white spruce (*Picea glauca* (Moench)), jack pine (*Pinus banksiana* Lamb.) and trembling aspen (*Populus tremuloides)* grown under elevated [CO_2]. Kimball (1983) reported that plants yield 33% higher when the [CO_2] is twice ambient level. Elevated [CO_2] has significant effects on resource allocation patterns (Larigauderie *et al.* 1988; Huang *et al.* 2007), as well as specific leaf area (SLA) (Zhang *et al.* 2006; Huang *et al.* 2007, Cao *et al.* 2008), branching patterns (Bazzaz 1990, Tissue *et al.* 1997) and stem elongation (Reekie *et al.* 1997, Ainsworth and Long 2005).

Light regime is another major driving variable for photosynthesis. It affects the biochemistry and morphology of leaves, resulting in changes in photosynthetic and respiratory rates that in turn affect plant growth rate (Awada and Redmann 2000). Light also influences plant response to [CO_2] and there is synergism between the two (Hopkins and

Hüner 2004). Depending on light conditions, elevated [CO_2] can influence the competitive ability of species by differentially altering their carbon balance (Bazzaz and McConnaughay 1992). Under high light conditions, plants are generally better able to take advantage of increased [CO_2] (Madsen 1974). At low light levels, however, light dependent reactions limit the rate of photosynthesis and increasing [CO_2] may not enhance the rate of photosynthesis as much. Despite the vital roles of [CO_2] and light in the growth, morphology and biomass of trees in the boreal forest, there is very little work on the interactive effects of [CO_2] and light on two of most important boreal tree species: black spruce and white spruce.

The boreal forest covers 11% of the earth's terrestrial surface (Bonan and Shugart 1989) with black spruce forests as the dominant cover type (Viereck and Johnston 1990), and has greater total ecosystem carbon content than any other forest biome (Gower *et al.* 1997). At the global scale, the boreal forest contains 800 Pg carbon (Apps *et al.* 1993), about half of the terrestrial biosphere's carbon stock (Schlesinger 1997). Climate change is expected to be more pronounced in the boreal region (Prentice *et al.* 2001). Thus, understanding the response of spruce forests to climate change is important to the understanding of the overall response of the boreal forest to climate change.

Black spruce and white spruce are congeneric species with similar growth, gross morphology and phenology (Patterson *et al.* 1997). However, black spruce is found mainly in lowland muskegs with poorly drained and low nutrient soils whereas white spruce generally grows on drier, nutrient rich upland sites (Vincent 1965, Larson 1980). White

spruce has greater demand for nutrient, moisture, light and other growth resources than associated conifers such as black spruce, making the range of land supporting its growth more limited with increasing climate severity (Nienstaedt and Zasada 1990). Black spruce, on the other hand, has broader distribution and can grow on sites that are not suitable for white spruce. However, both species are intermediate shade tolerant (Baker 1949, Haavisto and Jeglum 1995) with white spruce being less shade tolerant (Ritchie 1959, Nienstaedt and Zasada 1990). Vincent (1965) indicated that 25% of full sunlight does not significantly reduce height, shoot biomass, root biomass and total biomass of black spruce seedlings. Black spruce usually reaches photosynthetic light saturation at 25–50% full sunlight (Grossnickle 2000) while white spruce achieves maximum rate of photosynthesis at 40 – 60% of full sunlight (Man and Lieffers 1997) and maximum diameter and stem volume at full light (Lieffers and Stadt 1994). Minimum light levels for the survival of white spruce range from 8% (Lieffers and Stadt 1994) to 15% (Chen 1997) of full sunlight whereas black spruce can survive at even lower light levels (Haavisto and Jeglum 1995).

Increasing [CO_2] at low light may not directly improve the efficiency of light utilization because light is limiting photosynthesis (Walters and Reich 2000). However, elevated [CO_2] can suppress dark respiration and photorespiration and hence increase net photosynthesis and growth at irradiance levels above the compensation point and decrease the light compensation point for photosynthesis, consequently indirectly increasing the light use efficiency (Lambers *et al.* 1998). Therefore, it is hypothesized that CO_2 elevation stimulates the growth and biomass of black spruce and white spruce seedlings more (relatively) at lower than higher light levels. Teskey and Shrestha (1985) found that the photosynthesis of

more shade tolerant plants respond more positively than that of less shade tolerant plants to $[CO_2]$ elevation. It is further hypothesized that black spruce exhibits greater enhancement in growth and biomass under elevated $[CO_2]$ because of its greater shade tolerance than white spruce seedlings. To test the hypotheses, 1-year-old black spruce and white spruce was exposed to three light levels and two $[CO_2]$ over a period of 4.5 months under greenhouse conditions. This paper reports the morphological and biomass results.

Materials and Methods

Plant materials

One-year-old black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.) seedlings (Thunder Bay-Fort Frances provenance) were obtained from Hills Tree Nursery (Thunder Bay, Ontario). The seedlings were planted in 13.5 cm tall, 11 cm top diameter plastic containers containing a mixture of coarse premium grade vermiculite (Sungro Horticulture, Vancouver, Canada) and peat moss (50:50 v/v). The seedlings were relatively uniform in size (13 cm height) when the experiment started.

Experimental design

The experiment was a split-split plot design with CO_2 treatments as the whole plot, light treatments as sub-plots and the species as sub-sub-plots (App. 1). The treatments comprised of two CO_2 levels (360 μmol mol^{-1} and 720 μmol mol^{-1}) with two replications of each, three light levels (100%, 50% and 30% light) and two species (black spruce and white spruce)

and eight seedlings per treatment combination. The 192 seedlings used were selected from a total 600 to ensure uniformity in size and form.

The experiment was conducted in four greenhouses of the same design at Lakehead University's Thunder Bay-Ontario campus from January 15th 2007 to May 2nd 2007. Each CO_2 treatment was randomly assigned to two greenhouses and the three light levels were replicated in the greenhouses. The elevated [CO_2] was achieved using Argus CO_2 generators (Argus, Vancouver, BC, Canada) on cold days. On warm days, CO_2 was supplied from pressurised CO_2 tanks to avoid overheating. Neutral density shade cloths were used to provide the 50% and 70% shade levels (i.e. 50% and 30% light). All environmental conditions in the greenhouses were monitored and controlled using an Argus control system (Argus, Vancouver, BC, Canada). Light reaching plants grown at full light averaged 660μmol $m^{-2}s^{-1}$ on clear sunny days as measured with LI-190SA quantum sensor attached to LI-250A light meter (Licor Biosciences, Lincoln, USA). All the treatments were subjected to the same day and night temperatures (25 ± 2 °C and 15 ± 2 °C, respectively) with a 16-h photoperiod. The length of natural days was extended using high-pressure sodium lamps (P. L. Light Systems, Beamsville, Canada) in part of the experiment when the days were less than 16 hours. The moisture content of the growing medium was maintained at about 30% in all treatment combinations as measured using an HH2 Moisture Meter and ML2X ThetaProbe (Delta-T Devices, Cambridge, U. K.). A 55 ± 5% relative humidity was maintained. The seedlings were fertilised weekly with a solution of 150 mg/l N, 60 mg/l P, 150 mg/l K, 40 mg/l Mg, 80 mg/l Ca and 60 mg/l S. Nutrient levels of the growth

medium and irrigation water were measured and subtracted from the formulation at the beginning of the experiment (App. 2)

Measurements

Height, root collar diameter (RCD) and the number of first order lateral shoots (FOLS) were measured on all the seedlings after 2.5 months and 4.5 months of treatments. After 4.5 months, three seedlings from each treatment combination were randomly selected and harvested. Using an analytical balance (precision of 0.001g), fresh weight of shoots and roots were measured separately. The materials were then oven-dried at 70 °C for 48 h and dry mass was measured. Root mass ratio (RMR = root mass/total seedling mass), and shoot mass ratio (SMR = shoot mass/total seedling mass) were used as indices of biomass allocation. Projected area of current year needles was measured using a Regent WinSeedle System (Regent Instruments Inc., Quebec City, Quebec, Canada). The needles were oven-dried at 70 °C for 48 h to determine dry mass for calculating the SLA. Stem volume was calculated from height, RCD and mid-section stem diameter as follows:

$$\text{Stem volume} = 0.25(d_1 + d_2)^2 (\pi h),$$

where d_1 is RCD, d_2 is mid-section stem diameter, and h is height.

Statistical analysis

All data were examined graphically for normality of distribution (residual probability plots) and homogeneity of variance (scatter plots of residuals) using Data Desk 6.01 (Data Description Inc, New York, USA) before the analysis of variance was performed. The

effects of CO_2, light, species and their interactions were tested using the ANOVA linear model procedure in Data Desk. Scheffe's post-hoc tests were conducted when a main effect with more than two levels or interactions was significant ($p \leq 0.05$). Scheffe's post-hoc tests were made based on the rule that significant interactions override main effects and higher order significant interactions override lower level interactions.

Results

Height

CO_2 concentration and light had significant interactive effects on height after 2.5 months. The seedlings in full light and doubled [CO_2] were significantly higher than those in the other treatment combinations, but there were no significant differences among the other treatment combinations (Fig. 3.1a, App. 3). However, the significant CO_2 and light interaction became insignificant after 4.5 months while the main effects remained significant (Fig. 3.1b, App. 2). Light and species had significant interactive effects on height growth after 2.5 and 4.5 months. After 2.5 months of treatment, the height of black spruce grown under 100% light was the highest with no significant difference among the other treatment combinations (Fig. 3.1a). After 4.5 months of treatment, the height of black spruce at 100% light was still the highest, followed by white spruce at 100% light; there was no difference between the other treatment combinations (Fig. 3.1b).

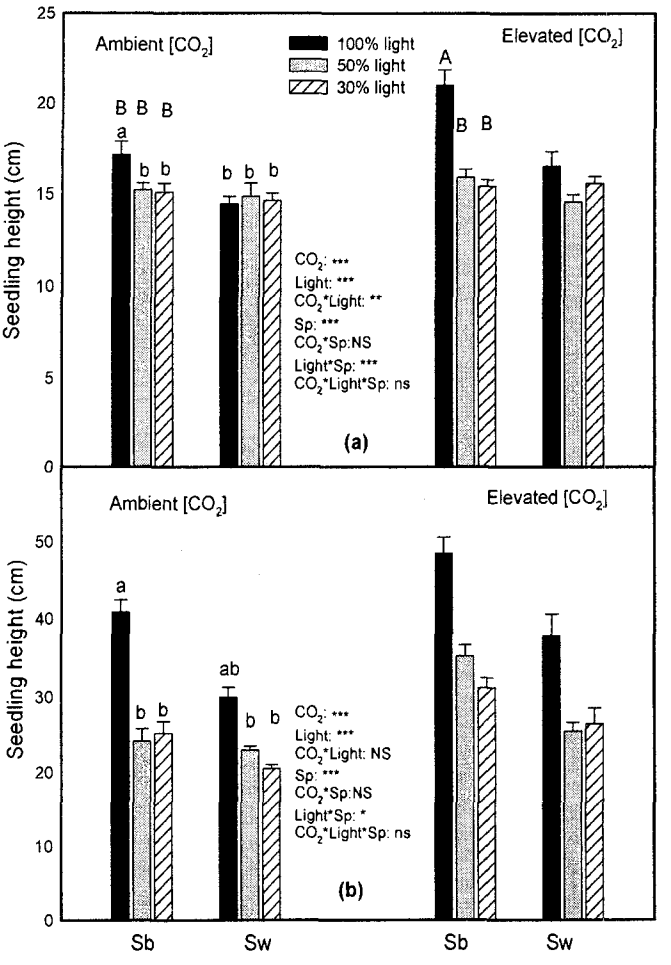

Fig. 3.1: Effects of $[CO_2]$ and light on the height of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In Fig. 3.1a, lower case letters represent light*species interactions and upper case letters represent CO_2*light interactions. In Fig. 3.1b, the letters represent light*species interactions. Means with the same letter or letters do not differ significantly ($p>0.05$) based on Scheffe's post-hoc test. Significance levels are represented by * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$) with ns representing not significant effects ($p>0.05$).

Root collar diameter

RCD after 2.5 months of treatment was significantly affected by [CO_2], light and species but no significant interactive effect occurred (Fig. 3.2a, App. 3). RCD was greater in seedling grown at 100% light than at the other two light levels (Fig. 3.2a). In addition, black spruce had significantly greater RCD than white spruce (Fig. 3.2a). RCD was significantly greater at elevated [CO_2] than at ambient [CO_2].

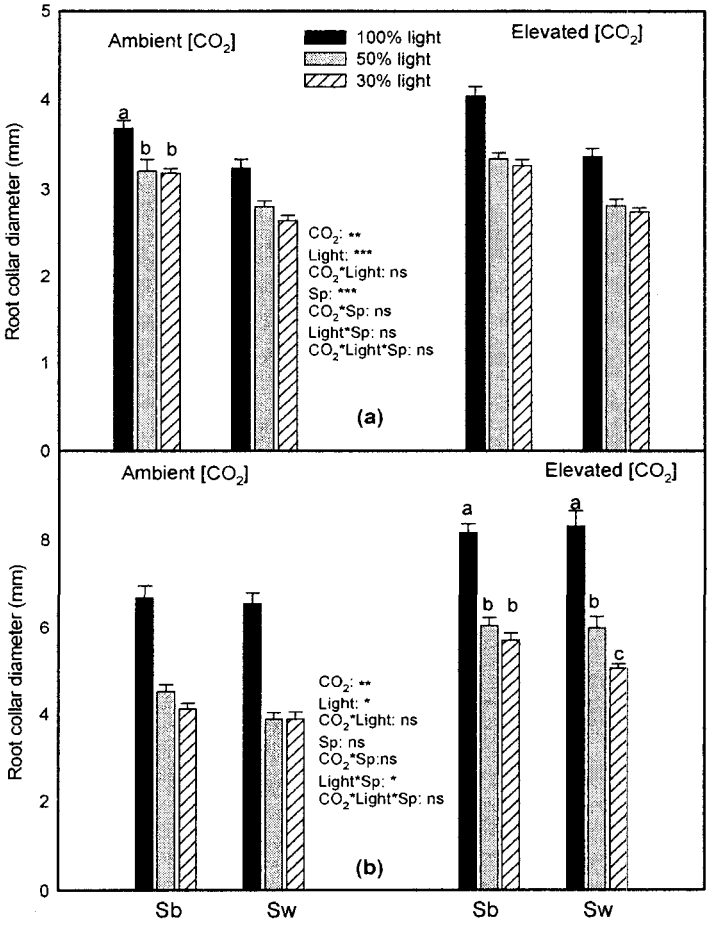

Fig. 3.2. Effects of [CO_2] and light on the root collar diameter of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In Fig. 3.2a, the letters represent light effects while in Fig. 3.2b, they represent light*species interactions. Other explanations are as in Fig. 3.1.

After 4.5 months of treatment, RCD was significantly greater at elevated [CO_2] than at ambient [CO_2] (Fig. 3.2b).The greatest RCD was observed in seedlings at 100% light in both species and there was only one significant difference among the other treatment combinations: white spruce at 30% light had the lowest RCD (Fig. 3.2b).

First order lateral shoots

[CO_2], light and species had significant interactive effects on the number of FOLS after 2.5 months of treatment (App. 3). Black spruce grown under 100% light and elevated [CO_2] had the highest number of FOLS, followed by black spruce at 100% light and ambient [CO_2] (Fig. 3.3a). The responses of black spruce grown under 50 and 30% light to CO_2 were similar to those for white spruce at 100% light at both [CO_2]. White spruce at 50 and 30% light at the two [CO_2] had the lowest number of FOLS (Fig. 3.3a). After 4.5 months of treatment, no significant interaction occurred (App. 3). However, seedlings at 100% light had greater FOLS than at the other light treatments and FOLS was significantly greater in black spruce than in white spruce (Fig. 3.3b, App. 3).

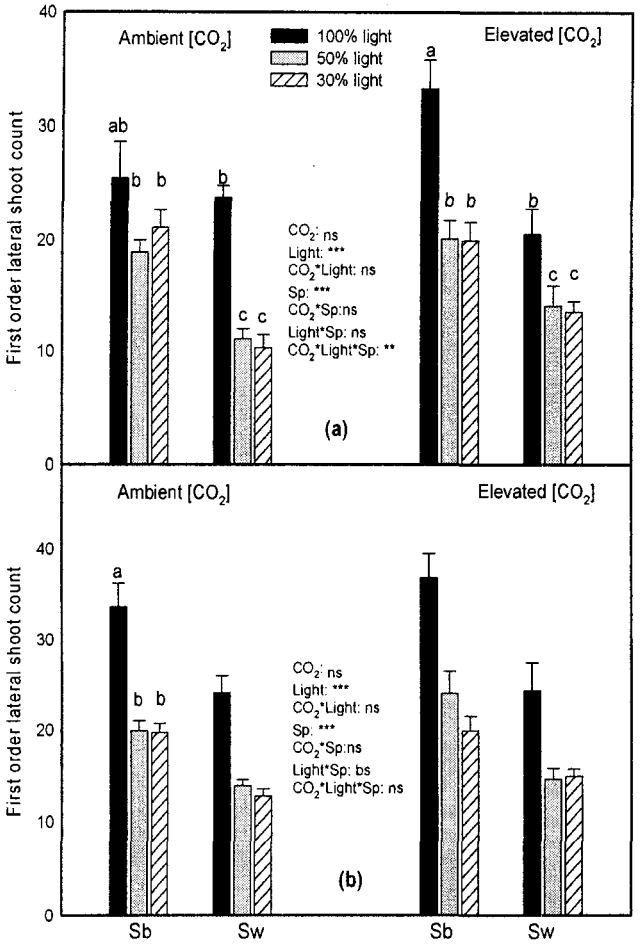

Fig. 3.3. Effects of $[CO_2]$ and light on the number of first order lateral shoot of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In Fig. 3.3a, the letters represents CO_2*light*species interactions while in Fig. 3.3b, they represent light effects. Other explanations are as in Fig. 3.1

Stem volume

$[CO_2]$ and light had significant interactive effects on stem volume. The greatest stem volume was observed in seedlings grown at 100% light under elevated $[CO_2]$ followed, sequentially, by seedlings grown at 100% light and ambient $[CO_2]$ and the shaded seedlings under elevated $[CO_2]$. No significant difference occurred in the shaded seedlings grown at ambient $[CO_2]$, where the lowest stem volume was recorded (Fig. 3.4a, App. 3). Light and

species also had significant interactive effects on stem volume (App. 3). Stem volume was greatest in black spruce grown under 100% light, followed by white spruce at 100% light and there was no significant difference between seedlings of either species at 50 and 30% light regimes (Fig. 3.4a).

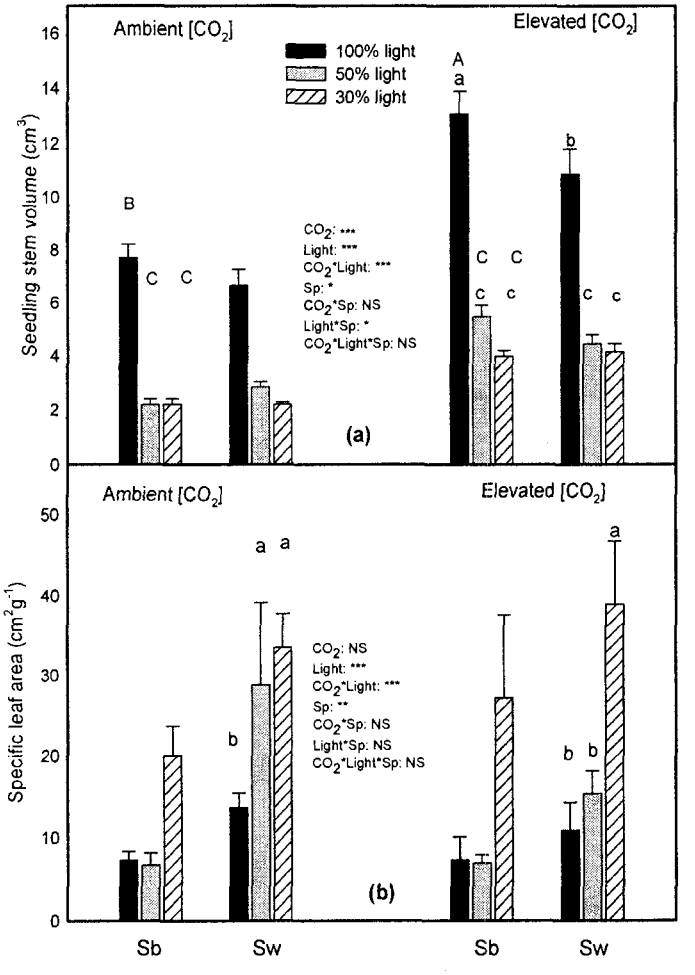

Fig. 3.4: Effects of $[CO_2]$ and light on the stem volume (4a) and specific leaf area (4b) of black spruce (Sb) and white spruce (Sw) seedlings after 4.5 months of treatment. In Fig. 3.4a, the lower case letters represent light*species interactions while upper case letters represent CO_2*light interactions. In Fig. 3.4b, the letters represent CO_2*light interactions. Other explanations are as in Fig. 3.1.

Specific leaf area (SLA)

[CO_2] and light had significant interactive effects on SLA (Fig. 3.4b, App. 3). Under both [CO_2], SLA generally decreased with increasing light. Seedlings grown at 50 and 30% light under ambient [CO_2] conditions, as well as seedlings grown at 30% light and elevated [CO_2] had the highest SLA (Fig. 3.4b). [CO_2] per se had no effect on SLA at the 100% regime. White spruce had significantly greater SLA than black spruce (Fig. 3.4b).

Biomass

The interactions of [CO_2], light and species significantly influenced total seedling fresh mass, dry mass, shoots fresh mass and shoots dry mass in the same pattern (App. 3). Black spruce grown at 100% light and elevated [CO_2] had the greatest total fresh and dry biomass, as well as shoot fresh and dry mass (Figs. 3.5a-d, respectively). This was followed by black spruce grown at 50% light under elevated [CO_2], white spruce at 100% light under both [CO_2] and black spruce at 100% ambient [CO_2]. Total and shoot biomass (both fresh and dry) of both species at 30% light and ambient [CO_2] were lower than those for both species at 30% light under elevated [CO_2].

The three factors also had significant interactions on root fresh mass and root dry mass Black spruce grown at 100% light under elevated [CO_2] had the greatest root fresh mass and this was followed by white spruce at 100% light under both CO_2 conditions (Fig. 6a). Under elevated [CO_2], the root fresh mass of either species was similar at 50 and 30% light. Within ambient [CO_2] the root fresh mass of either at 50 and 30% light were similar but lower than at elevated [CO_2]. Black spruce grown at elevated [CO_2] and 100% light had the greatest

root dry mass, followed by white spruce at 100% light at both [CO_2], and these were significantly greater than root dry mass of black spruce grown at ambient [CO_2] under 100% light. Root dry mass was lowest in black spruce exposed to elevated [CO_2] and 30% light (Fig. 3.6b).

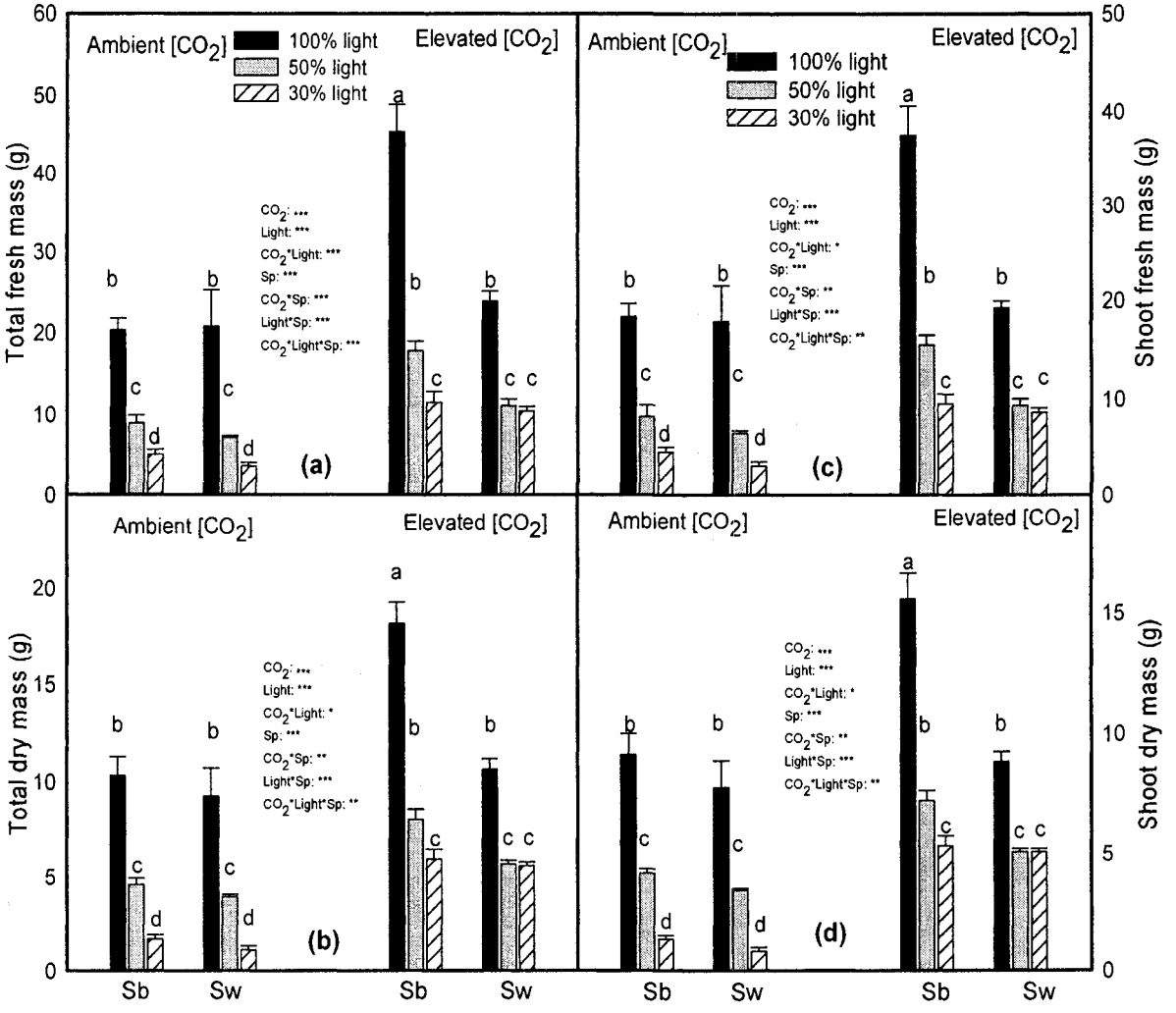

Fig. 3.5: Effects of [CO_2] and light on the total fresh (a), total dry (b), shoot fresh (c) and shoot dry (d) mass of black spruce (Sb) and white spruce (Sw) seedlings after 4.5 months of treatment. The letters represent CO_2*light*species interactions in Figs. 3.5a - d. Other explanations are as in Fig. 3.1.

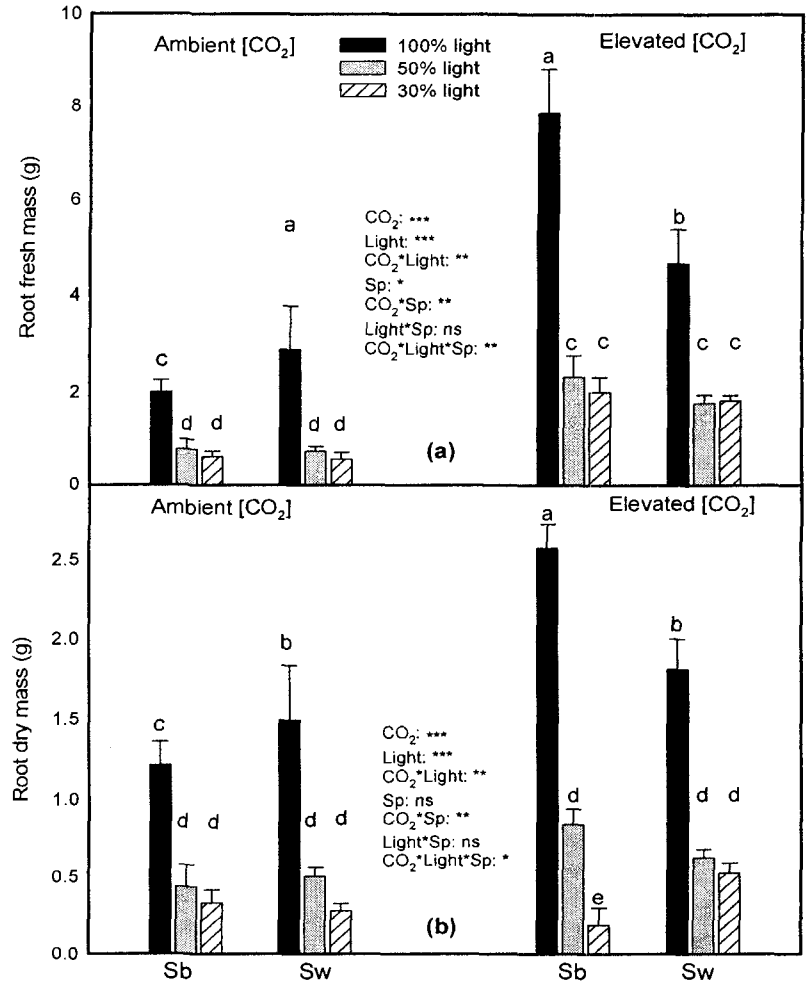

Fig. 3.6: Effects of [CO_2] and light on the fresh (a) and dry (b) root biomass of black spruce (Sb) and white spruce (Sw) seedlings after 4.5 months of treatment. The letters represent CO_2* light*species interactions in both Figs. 3.6a and 3.6b. Other explanations are as in Fig. 3.1

Biomass ratios

[CO_2] and light had significant interactive effects on SMR (App. 3). SMR decreased with decreasing light under ambient [CO_2], whereas the opposite occurred in seedlings grown at elevated [CO_2]. At ambient [CO_2], seedlings grown at 100 and 50% light had statistically higher SMR, whereas under elevated [CO_2], the highest SMR was recorded in seedlings

grown at 50 and 30% light. Seedlings grown at 30% light under ambient [CO_2] and 100% light under elevated [CO_2] had the lowest SMR (Fig. 3.7a).

CO_2, light and species had significant interactive effects on RMR (App. 3). White spruce grown at 30% light under ambient [CO_2] had the greatest RMR. There was no difference between ambient and elevated [CO_2] in the RMR of black spruce grown at 30 and 100% light respectively, or white spruce grown at 100% light (Fig. 3.7b). No significant difference occurred in other treatment combinations which had the lowest RMR (Fig. 3.7b).

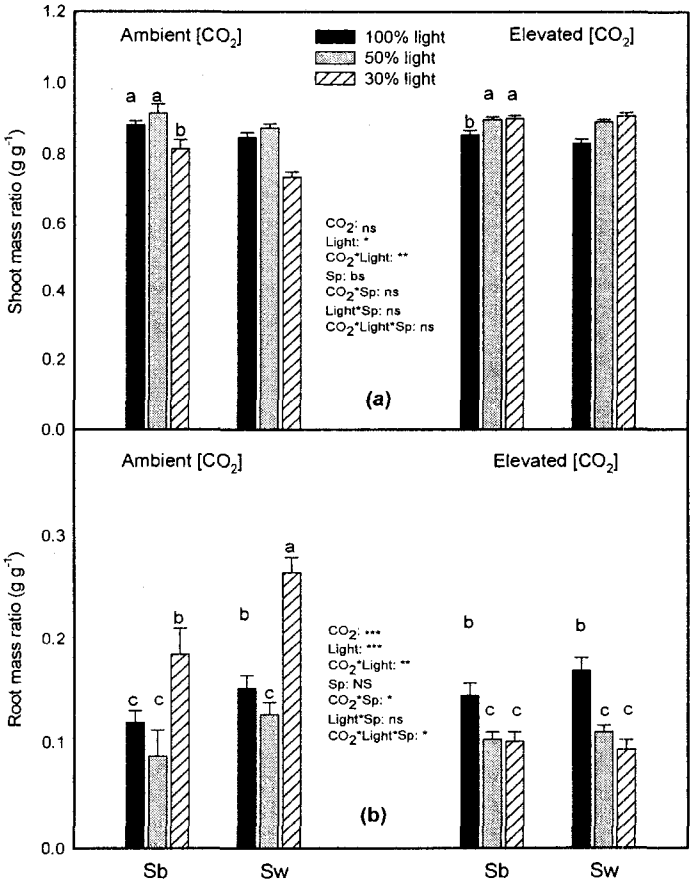

Fig. 3.7: Effects of [CO_2] and light on the shoot mass ratio (a) and root mass ratio (b) of black spruce (Sb) and white spruce (Sw) seedlings after 4.5 months of treatment. In Fig. 3.7a, the letters represent CO_2*light interactions while in Fig. 3.7b, they represent CO_2*light*species interactions. Other explanations are as in Fig. 3.1

[CO_2] and light, and CO_2 and species had significant interactive effects on the shoot to root mass ratio (SRR) (App. 3). At ambient [CO_2], SRR decreased with decreasing light whereas under elevated [CO_2], SRR increased with decreasing light. At ambient [CO_2], the SRR of seedlings at 100 and 50% light was significantly higher than that in seedlings at 30% light under ambient [CO_2] (Fig. 3.8). However, under elevated [CO_2], the SRR of seedlings at 50 and 30% light was significantly higher than the SRR of seedlings at 100% light. At elevated [CO_2], there was no significant difference in SRR, whereas at ambient [CO_2] white spruce had significantly reduced SRR (Fig. 3.8).

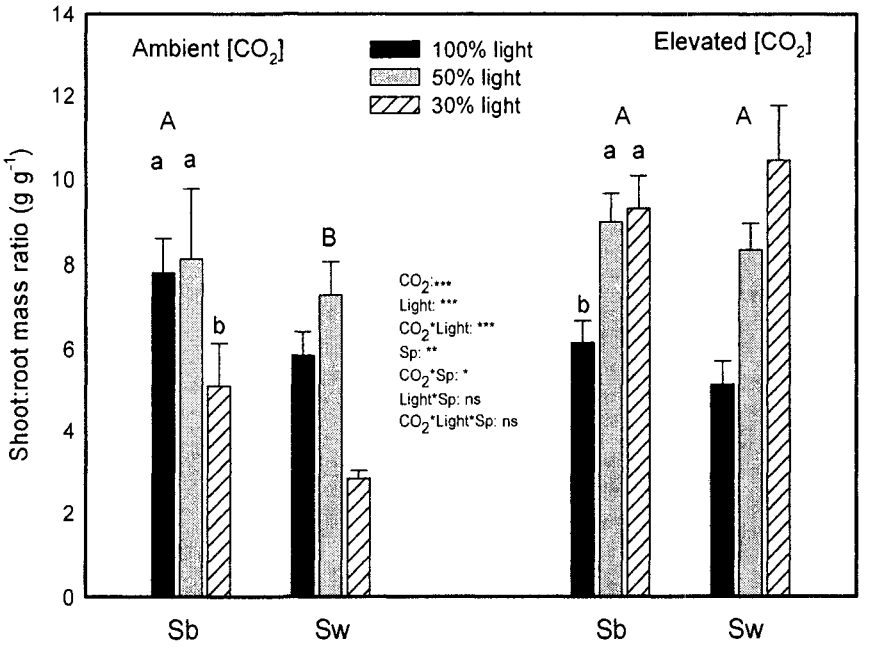

Fig. 3.8: Effects of [CO_2] and light on the SRR of black spruce (Sb) and white spruce (Sw) seedlings after 4.5 months of treatment. The lower case letters represent CO_2*light interactions while upper case letters represent CO_2*species interactions. Other explanations are as in Fig. 3.1.

Discussion

The data obtained in the present study supports the hypothesis that CO_2 enhancement of growth and biomass in the two species was relatively greater at lower light levels than at higher light. Despite the greater absolute increases found at higher light (eg: Figs. 3.1b, 3.5a-5d), the far greater relative increases at lower lights suggest that increases in CO_2 concentration in the atmosphere will likely increase the shade tolerance and competitiveness of the two species under shaded conditions. Consequently, these species will likely be able to grow longer and faster under the shade of the existing forest canopy. Thus, understory regeneration and uneven-aged silvicultural systems will likely become more practical in the future. The replacement of clearcut silvicultural system for even-aged stands by uneven-aged management systems will represent a major advance in the silviculture of the boreal forest. Well structured uneven-aged stands are generally more productive and more sustainable, particularly on a small land base, than even-aged stands (Smith *et al.* 1997). This result can have important implications for forest management and silviculture under future climatic conditions.

$[CO_2]$ and light had significant interactive effects on seedling height after 2.5 months of treatments. Under the 100% light regime, the seedlings were able to take advantage of the elevated $[CO_2]$, resulting in greater seedling height at the first measurement. The CO_2-light synergy has also been reported in other studies (Kerstiens 1998; Hopkins and Hüner 2004). In the presence of sufficient light, plant growth is generally enhanced (Reekie *et al.* 1997; Zebian and Reekie 1998; Grechi *et al.* 2007) because plants are better able to use increased CO_2 in terms of absolute responses (Madsen 1974). Zebian and Reekie (1998) reported that

the combination of increased CO_2 and high light enhance the seedling height growth of *Sinapis alba* L., *Medicago sativa* L., *Gypsophila paniculata* L. and *Picea abies* (L.) Karsten more than did either treatment alone. In this study however, the CO_2-light interaction became insignificant after 4.5 months of treatment (Fig. 3.1b), suggesting that the height responses became uniform in the statistical sense among different light treatments. Since the seedlings under shade were smaller at 2.5 months, the disappearance of the differences suggests greater relative responses at lower light. Thus, the height data also lends to support to our first hypothesis. The greater relative responses at lower light conditions will likely increase the competiveness of the species under shaded conditions. Khan *et al.* (2000) also found that Douglas-fir, ponderosa pine, western hemlock and western red cedar grown at 25% light have the greatest height while seedlings at 100% light have the lowest height.

Black spruce responded more dramatically to CO_2 elevation than its congeneric counterpart. This result supports our second hypothesis that the more shade tolerant species (i.e., black spruce) is more responsive to elevated CO_2. Poorter and Werger (1999) found similar results in *Cecropia ficifolia* Warb. ex Snethl, *Bellucia pentamera* Naudin, *Tachigali* sp. *Cariniana micrantha* Duke, *Capirona decorticans* Spruce *and Theobroma speciosum* Willd. ex Spreng. Plants that have similar morphological features can respond differently to light and CO_2 treatments (Johnsen and Major 1998; Grechi *et al.* 2007) and the response to [CO_2] can change with light conditions (Hättenschwiler and Körner 2000). These results suggest that more shade tolerant species will likely benefit more from increases in the atmospheric CO_2. Faster growth of understory species or advance growth can accelerate the process of succession in the forest.

Seedling height is generally positively correlated to whole seedling photosynthetic capacity and transpiration efficiency (Armson and Sadreika 1974; Thompson 1985). Seedlings that are better able to take advantage of elevated [CO_2] will likely be more competitive under future climatic conditions associated with increasing atmospheric [CO_2]. Johnson and Major (1998) found that black spruce seedlings grown under elevated [CO_2] have greater height and RCD and subsequently have a greater survival rate and better growth when planted in the field than those grown under ambient [CO_2]. Such differences are still evident 15 years after planting. Similar results are found with white spruce 10 years after field planting (Mullin and Svanton 1972).

The diameter growth of the two species responded differently to light treatment. In white spruce, the diameter generally decreased with decreasing light levels. In black spruce, however, there was no significant difference in the RCD of seedlings exposed to 50 and 30% light, although 100% light still produced the largest RCD. After 2.5 months, RCD of black spruce was 18% higher than that of white spruce and the margin of increase dropped to 5% after 4.5 months. Regardless of the light level, elevated [CO_2] increased RCD of black spruce and white spruce by 30 and 35%, respectively, after 4.5 months, an indication that increased [CO_2] would stimulate greater tree growth even at lower light levels above the minimum required for survival.

The synergetic effect of [CO_2] and light was also evident in stem volume measurement, i.e., the greatest stem volume was achieved under elevated [CO_2] and highest light regime. The trends of the synergistic effect were similar for seedling height, RCD and stem volume. The

relative enhancement by elevated [CO_2] on stem volume was however different from the gross enhancement. For example, elevated [CO_2] increased stem volume by 67, 98 and 84% in seedlings grown at 100, 50 and 30% light, respectively. In terms of species response, the relative enhancement by elevated [CO_2] was 86 and 66% for black spruce and white spruce, respectively. The greater relative enhancement at low light seems to suggest an enhancement of light use efficiency in shaded seedlings (Lambers *et al.* 1998). The greater enhancement at the low light levels may also be attributed to a reduction in respiration (Lambers *et al.* 1998), while the relatively greater enhancement of black spruce supports the findings of Teskey and Shrestha (1985) that more shade tolerant species respond more positively to [CO_2] elevation than less shade tolerant plants.

The number of FOLS was generally less sensitive to the treatments than the other morphological parameters, e.g., height and diameter, especially during the second measurement. Furthermore, it was more responsive to light than [CO_2]. The lack of significant response of FOLS to [CO_2] in this study was in contrast to the finding of Johnsen and Major (1998) that elevated [CO_2] increases the number of branches in black spruce seedlings by 78%. According to Lambers *et al.* (1998), shade intolerant plants generally do not respond to external environmental stimuli by varying the number of branches because of strong apical dominance. On the other hand, shade tolerant species do tend to vary the number of branches in response to environmental conditions (Poorter and Werger 1999).

There were significant interactive effects of [CO_2] and light on the SLA of both species. While the elevated [CO_2] appeared to have decreased SLA at all three light levels, the effect

was statistically significant only in the 50% light treatment. Our findings are in contrast to those of Zebian and Reekie (1998), Zhang *et al.* (2006) and Grechi *et al.* (2007) where neither [CO_2] nor its interaction with light affects SLA, but the negative effect of CO_2 elevation on SLA is common in trees (Pettersson and Mcdonald 1992, Wolfe *et al.* 1998). Changes in leaf structure, anatomy and/or accumulation of soluble carbohydrates can result in changes in SLA (Lambers *et al.* 1998) and elevated [CO_2] generally cause accumulation of carbohydrates in leaves, reducing SLA (Pettersson and Mcdonald 1992). Plants growing under shade conditions tend to increase their leaf area to acquire more light photons (Lambers *et al.* 1998, Zebian and Reekie 1998, Poorter 2001, Grechi *et al.* 2007) Conifers also adopt a strategy to increase their photosynthetic capacity by decreasing SLA with increasing light levels (Jordan and Smith 1993). However, the exact mechanisms for the differences found in this study are unknown.

There were significant three-way interactions among [CO_2], light and species in all biomass variables in this study, including total seedling, shoot and root biomass (both fresh and dry. The relative enhancement of seedling fresh and dry biomass by elevated [CO_2] increased as light levels decreased. Elevated [CO_2] increased total fresh mass by 68, 79 and 318% and total dry mass by 47, 61 and 318% at 100, 50 and 30% light, respectively. The explanation for the greater enhancement at lower light is similar to the ones outlined for RCD. The biomass enhancement levels were greater in black spruce than in white spruce (for example, total fresh mass was 165% higher in black spruce while total dry mass was 75%). While the total and shoot biomass of black spruce decreased with decreasing light levels under both the ambient and elevated [CO_2], such a pattern was true for white spruce only under the

ambient [CO_2]. Under the elevated [CO_2], in contrast, there were no significant differences in either total or shoot biomass in white spruce. While the above trends appear to hold for root biomass, the differences between the two shaded levels generally lack statistical significance, with the exception of black spruce under elevated [CO_2]. There are two possible explanations for the general lack of significant difference in seedlings grown under shade conditions. First, root biomass of seedlings grown at 50 and 30% light was very small in comparison to that of shoot or total biomass and the differences were even smaller than those of total or shoot biomass. Second, since the rate of shoot growth determines the success of a tree in competition for light, the resource allocation to roots was kept at a bare minimum under both 30% and 50% light. However, the elevated [CO_2] did increase the sensitivity of root biomass to light treatments in black spruce.

Elevated [CO_2] decreased SMR at 100% light, but increased it at 30% light. By contrast, the RMR at 30% light was the greatest under the ambient [CO_2] but the smallest under elevated [CO_2]. At 100% light, on the other hand, the RMR was intermediate under the ambient [CO_2] but became the highest under the elevated [CO_2]. As a consequence of the different responses in RMR and SMR, the shoot to root ratio was lowest at 30% light under ambient [CO_2] and 100% light under the elevated [CO_2]. In other words, the CO_2 elevation appeared to cause a reduction in resource allocation to the shoot under the high light regime but an increase under the low light condition. This shift in resource allocation in response to changes in [CO_2] could potentially influence the relative competitiveness of the species under different light conditions in the future climate when the atmospheric [CO_2] will be substantially higher than it is today. The findings in this study are in general agreement

with some studies (Duff *et al.* 1994, Prior *et al.* 1997, Grechi *et al.* 2007), but in contrast to others in which [CO_2] does not affect mass ratios (Tissue *et al.* 1997, Zhang *et al.* 2006).

Regenerating black spruce and white spruce can be challenging with increasing emphasis on smaller clearcuts and uneven-aged forest stand management. Understanding the morphological and physiological requirements of seedlings is essential for proper management strategies. The continuous increase in atmospheric [CO_2] makes the situation even more important, especially in the boreal forest where the impacts of CO_2-mediated climate change will be most prominent. Regardless of the light level, elevated [CO_2] increased height and diameter growth by 21 and 16% and 20 and 23%, respectively, in black spruce and white spruce. Total biomass was also 94 and 54% higher under elevated [CO_2] in black spruce and white spruce, respectively, with some shifts in resource allocation between above and below ground parts. The shift in resource allocations in response to elevated [CO_2] could have significant implications to future regeneration efforts. While some parameters such as height and FOLS were enhanced relatively greater by elevated [CO_2] at high light level, total fresh and dry biomass, as well as RCD, were relatively more enhanced at the lower light levels. The presumed enhancement in light use efficiency under shaded conditions suggests that uneven-age silvicultural systems for managing black and white spruce would be more feasible under future climatic conditions associated with increases in atmospheric [CO_2]. However, longer term research is warranted to better understand the implications.

Chapter 4

Physiological responses of black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.) seedlings to elevated carbon dioxide concentration under different light conditions

Abstract

The physiological responses of 1-year old black spruce and white spruce seedlings grown at 360 and 720 μmol mol^{-1} [CO_2] under 100, 50 and 30% light were investigated. CO_2 elevation enhanced net photosynthesis by an average of 40, 82 and 61% at 100, 50 and 30% light respectively. Seedlings grown at elevated [CO_2] had a 13 and 17% reduction in CO_2 compensation point (Γ) after 2.5 and 4.5 months respectively. The Γ decreased with decreasing light especially in black spruce. CO_2 elevation reduced day-time dark respiration rate and the magnitude of reduction was higher in white spruce than black spruce and at lower light levels. Stomatal conductance (g_s) was 43% lower at elevated [CO_2]. CO_2 elevation reduced transpiration rate (E) by 18 and 14% after 2.5 and 4.5 months, respectively, especially in black spruce. E decreased with decreasing light. After 2.5 months, maximum carboxylation rate (Vc_{max}) increased with increasing light in black spruce, and white spruce's response at 100% light was equal to black spruce's 50% light. CO_2 elevation increased light saturated electron transport rate (J_{max}) after 4.5 months and total electron transport rate (J_t) and the increase was highest at 100% light. CO_2 elevation reduced light compensation point and increased saturation point for photosynthesis.

Keywords: boreal forest, CO_2-light-species interaction, photochemical efficiency, respiration, ecophysiological response, water use efficiency.

Introduction

Atmospheric carbon dioxide concentration ($[CO_2]$) has increased continually since the pre-industrial revolution (Pearson and Palmer 2000, Prentice *et al.* 2001, IPCC 2007). Elevated $[CO_2]$ is expected to have great impact on the physiology, growth and biomass production of plants (Curtis and Wang 1998, Ward and Strain 1999). Many researchers (e.g., Cure and Acock 1986, Wuebbles *et al.* 1999, Lindner 2000, Ainsworth and Long 2005, Zhang and Dang 2005, Zhang and Dang 2006, Zhang *et al.* 2006, Huang *et al.* 2007) have reported enhanced photosynthesis and biomass production in response to increased $[CO_2]$. Elevated $[CO_2]$ is known to reduce stomatal conductance and transpiration, and increase photosynthetic rate leading to improved water use efficiency, greater light use efficiency (Drake *et al.* 1997, Körner 2000). By increasing the carboxylation rate of Rubisco and inhibiting the competitive process of oxygenation, CO_2 elevation is able to increase the photosynthetic rate (Drake *et al.* 1997). For example, Ceulemans and Mousseau (1994) found that doubled atmospheric $[CO_2]$ increased leaf level photosynthesis by approximately 40% in conifers and 60% in hardwood trees. Zhang and Dang (2007) reported an enhancement of biomass production in black spruce (*Picea mariana* [Mill.] B. S. P), white spruce (*Picea glauca* (Moench)), jack pine (*Pinus banksiana* Lamb.) and trembling aspen (*Populus tremuloides)* grown under elevated $[CO_2]$. Saxe *et al.* (1998) reported a 67% net photosynthesis enhancement in field grown *Pinus taeda* L. exposed to elevated $[CO_2]$. Ainsworth and Long (2005) reported 31% and 21% enhancement in light-saturated photosynthetic rate and total daily photosynthetic carbon assimilation, respectively, in plants exposed to elevated $[CO_2]$ in a free-air carbon dioxide experiment (FACE). Elevated $[CO_2]$

is also known to increase apparent quantum yield and decrease rates of dark respiration (Tesky and Shrestha 1985, Saxe *et al.* 1998, Hymus *et al.* 2001).

Light affects the biochemistry and morphology of leaves, resulting in changes in rates of photosynthesis and respiration that in turn affect plant growth rate (Awada and Redmann 2000). Light also modifies plant response to [CO_2] and there can be synergism between the two (Saxe *et al.* 1998, Hopkins and Hüner 2004). Depending on the flux density of photosynthetically active radiation, elevated [CO_2] can influence the competitive ability of species by differentially altering their carbon balance (Bazzaz and McConnaughay 1992). For example, while no significant difference occurred in the carboxylation efficiencies of American beech, white oak, yellow poplar and northern red oak grown at high light under elevated [CO_2], significant differences occurred in these plants at low light (Teskey and Shrestha 1985). The synergism between [CO_2] and light ensures that plants under high light conditions are also able to take advantage of increased [CO_2] (Madsen 1974). At higher [CO_2], plants have high carboxylation efficiency at low light, resulting in greater photosynthetic rates (Teskey and Shrestha 1985). In a related study (Marfo and Dang 2008), it was found that although biomass and biomass components increased with increased light under elevated [CO_2], the relative increase was greater at low light levels. Elevated [CO_2] is known to reduce the light compensation point for photosynthesis (Saxe *et al.* 1998), allowing plants to improve light use efficiency and thus to be able to grow in deeper shade (Körner 2006).

Despite the important effects of [CO_2] and its interactions with light, there exists a large knowledge gap on their effects on the physiology of black spruce and the congeneric white spruce (Marfo and Dang 2008). Such information is critical for a better understanding of potential impact of increasing atmospheric [CO_2] on the physiological performance and regeneration of the boreal forests under different silvicultural systems, such as clearcut versus uneven-aged selection cut systems in future climatic conditions.

Of all forest biomes on earth, elevated [CO_2]-mediated climate change is expected to have the greatest impact on the boreal forest (Prentice *et al.* 2001). The boreal forest is the largest terrestrial ecosystem, covering 11% of the earth's terrestrial surface (Bonan and Shugart 1989) and contains 800 Pg carbon (Apps *et al.* 1993), about half of the terrestrial biosphere's carbon stock (Schlesinger 1997). Black spruce is a dominant species in the boreal forest (Viereck and Johnston 1990). Black spruce forests have greater total ecosystem carbon content than any other forest biome (Gower *et al.* 1997).

Black spruce and white spruce are intermediate shade tolerant species (Baker 1949, Haavisto and Jeglum 1995), with white spruce being less shade tolerant (Ritchie 1959, Nienstaedt and Zasada 1990). Black spruce can reach growth and biomass production at 25% of full sunlight (Vincent 1965) and reach photosynthetic saturation at between 25 - 50% of full sunlight (Grossnickle 2000), while white spruce seedlings reach photosynthetic saturation at 40 – 60% of full sunlight (Man and Lieffers 1997). Minimum light levels for the survival of white spruce range from 8% (Lieffers and Stadt 1994) to 15% (Chen 1997) of full sunlight while black spruce can survive at 10% levels (Haavisto and Jeglum 1995).

These two congeneric species have similar growth rate, gross morphology and phenology (Dudley, 1996). However, black spruce can grow on lowlands and low nutrient soils, while white spruce usually grows on richer upland sites (Larson 1980). Additionally, white spruce is reported to have greater demand for nutrients, moisture, light and other growth resources than associated conifers such as black spruce, making the range of suitable sites more limited with increasing climate severity (Nienstaedt and Zasada 1990).

Since an increase in $[CO_2]$ can suppress Rubisco oxygenation and thus increase energy partitioning to carboxylation from the photosynthetic electron chain (Drake *et al.* 1997, Lambers *et al.* 1998), it is hypothesised that black spruce and white spruce seedlings grown at elevated $[CO_2]$ will have greater rates of net photosynthesis and lower rates of dark respiration than at ambient $[CO_2]$. Under elevated $[CO_2]$, higher carboxylation efficiency at low light positively correlates with higher rates of net photosynthesis (Teskey and Shrestha 1985), and respiration at lower light levels is reduced (Drake *et al.* 1997, Lambers *et al.* 1998). It is expected that the relative photosynthetic enhancement by increased $[CO_2]$ would be greater at lower light levels. Because more shade tolerant species tend to respond relatively more positively to elevated CO_2 (Teskey and Shrestha 1985) and black spruce is more shade tolerant than white spruce (Ritchie 1959, Nienstaedt and Zasada 1990), it is further hypothesized that the photosynthetic rate of black spruce would be enhanced by elevated $[CO_2]$ to a greater extent than white spruce. If elevated $[CO_2]$ is able to increase both photosynthetic rate as hypothesised and reduce stomatal conductance, then there would be corresponding decrease in transpiration rate and an increase in instantaneous water use

efficiency. This is because as stomatal conductance is reduced, the water potential within the plant is conserved (Drake *et al.* 1997, Körner, 2006).

Materials and methods

Plant materials

One-year old seedlings of black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.) were obtained from Hills Tree Nursery (Thunder Bay, Ontario). The seedlings were selected such that they were uniform in size (13 ± 1 cm high) and form at the beginning of the experiment. The seedlings were planted in 13.5 cm tall, 11 cm top diameter Kord plastic containers. The growing medium was a coarse premium grade vermiculite-peat moss mixture (50:50, v/v).

Experimental design

The experiment was a split-split plot design (App. 1) with the CO_2 concentration as the whole plot, light as the sub-plot and the species as the sub-sub plot. The treatment combination comprised two CO_2 levels with two replications each (360 and 720 μmol mol^{-1} for ambient and elevated [CO_2] respectively), three light levels (100%, 50% and 30% light) and the two species (black spruce and white spruce). There were 8 seedlings per treatment combination, giving a total of 192 seedlings. The study was conducted in four adjacent greenhouses of similar design at the Lakehead University Thunder Bay campus.

On days with greenhouse air temperatures lower than the growth temperature, Argus CO_2 generators (Argus, Vancouver, BC- Canada) were used to elevate the carbon dioxide. On hot days, the CO_2 was elevated from pressurised CO_2 tanks to avoid heat generation by the

CO_2 generators. Neutral density shade cloths were used to provide 50% and 70% shade, resulting in 50% and 30% light respectively. Light measurements were done on clear sunny days using LI-190SA quantum sensor integrated with LI-250A light meter (Li-cor Biosciences, Lincoln, USA) and readings at full light (100% light) were approximately 660 μmol $m^{-2}s^{-1}$. An Argus control system (Argus, Vancouver, BC, Canada) was used to monitor and control all the environmental conditions in the four greenhouses. Day and night temperatures were maintained at 25 ± 2°C and 15 ± 2°C, respectively, with a 16-hour photoperiod. The natural light was supplemented artificially on cloudy days, early mornings and late evenings with high-pressure sodium lamps. Growth medium moisture content was maintained at 30% in all treatment combinations with measurements taken with an HH2 Moisture Meter and ML2X ThetaProbe (Delta-T Devices, Cambridge, U. K.). Relative humidity in the greenhouses was maintained at 55± 5% during the 4.5 month experiment. All the seedlings were fertilised weekly with a solution of 150 mg/l N, 60 mg/l P, 150 mg/l K, 40 mg/l Mg, 80 mg/l Ca and 60 mg/l S. The nutrient levels of growth media and irrigation water were measured and subtracted from total fertiliser required to complete the chemical formulation (App. 2).

Gas exchange and chlorophyll fluorescence measurements

Foliar gas exchange was measured using a PP-Systems Ciras-1 open gas exchange system (Hitchin, Herefordshire, U. K.) with a Parkinson leaf cuvette for conifers. Environmental conditions in the cuvette were controlled automatically as follows: temperature 25°C, Rh 50%, photosynthetically active radiation (PAR) flux density 800 μmol m^{-2} s^{-1}. The light was supplied from the cuvette's built-in lamp. *A-C*$_i$ curves (CO_2 assimilation rate, *A*, versus

calculated sub-stomatal [CO_2], C_i) were measured sequentially at 50, 150, 250, 360, 450, 550, 700, 900 and 1100 μmol mol^{-1} [CO_2]. Light response curves were measured at 1100, 800, 650, 550, 450, 300, 150, 80, 40 and 10 μmol m^{-2} s^{-1} PAR flux density. Chlorophyll fluorescence was measured with a FMS-2 portable pulse-modulated fluorometer (Hansatech Instruments, Norfolk, U. K) simultaneously with gas exchange measurements. The fluorometer and gas exchange system were integrated to permit simultaneous measurement.

All measurements were taken between 0900 and 1500 hours on sunny days to minimise diurnal and between-day effects. Three plants from each treatment combination were randomly selected for the measurements. All measurements were taken on fully expanded current year needles near the apex of the seedlings, 2.5 and 4.5 months into the treatment. The seedlings were maintained in their original treatment conditions during the measurement. Each curve measurement cycle took approximately 40 minutes to complete.

Gas exchange parameters are expressed on a projected leaf area basis. Leaf area was measured using the Regent Winseedle system (Regent Instruments, Québec City, QC, Canada). Net photosynthesis at the growth concentration was obtained by interpolating the two measured photosynthetic rates immediately below and above 360 and 720 μmol mol^{-1} (ambient and elevated [CO_2], respectively) on the *A-Ci* curves. The corresponding stomatal conductance and transpiration rates were used for the analysis. Instantaneous water use efficiency (IWUE) was calculated as: IWUE = A/E, where A is the photosynthetic rate and E is the transpiration rate.

The maximum carboxylation rate of Rubisco (Vc_{max}), light-saturated electron transport rate (J_{max}), rate of triose phosphate utilisation (TPU) and CO_2 compensation point (Γ) were calculated using the mechanistic model of A-Ci analysis in Photosyn Assistant (Dundee Scientific, Scotland, U. K.). The mechanistic analysis was based on the model proposed by Farquhar *et al.* (1980), as subsequently modified by von Caemmerer and Farquhar (1981), Sharkey (1985), Harley and Sharkey (1991) and Harley *et al.* (1992). Michaelis-Menten constants of Rubisco for CO_2 (K_c) and O_2 (K_o) and the specificity factor for Rubisco (τ) and their temperature dependencies were adopted from Wullschleger (1993) and Harley *et al.* (2007). The A-C_i empirical model in the software was used to determine carboxylation efficiency (CE), i.e., the initial slope of the A-C_i curve. According to Farquhar *et al.* (1980) and Genty *et al.* (1989), simultaneous gas exchange and chlorophyll fluorescence are related as follows:

$$J_t = \Phi\ 0.5(\text{PPFD})(0.84) \quad (1)$$

$$J_t = 4(V_c + V_o) \quad (2)$$

$$P_n = Vc - 0.5Vo - R_{day} \quad (3)$$

Where Φ = the actual photochemical efficiency of photosystem II, where Φ_{PSII} = ((Fḿ - F)/Fḿ or ΔF/ Fḿ), J_t = apparent rate of electron transport, PPFD = PAR flux density, P_n = rate of net photosynthesis, R_{day} = rate of dark respiration during photosynthesis, V_c = carboxylation rate of Rubisco, V_o = oxygenation rate of Rubisco.

According to Epron *et al.* (1995), J_t, P_n and R_{day} are related to the partitioning of total electron flow between carboxylation (J_c) and oxygenation (J_o) as follows:

$$J_c = 1/3\ (J_t + 8(P_n + R_{day})) \quad (4)$$

$$J_o = 2/3(J_t - 4(P_n + R_{day})) \quad (5)$$

The light-response curve analysis in Photosyn Assistant (Dundee Scientific, Scotland, U. K.) was used to estimate light compensation point (LCP) and light saturation point (LSP).

Statistical analysis

The data were examined graphically for normality (using residual probability plots) and homogeneity of variance (using scatter plots of residuals) in Data Desk 6.01 (Data Description Inc, New York, USA) before the analysis of variance was performed. All of the data met the ANOVA assumptions. CO_2, light and species were considered as fixed factors in the ANOVA. The effects of CO_2, light, species and their interactions were tested using the ANOVA procedure in the Data Desk. Scheffe's post-hoc tests were conducted when light and/or interactions were significant ($p \leq 0.05$). Scheffe's post-hoc tests were made based on the rule that significant interactions override main effects and higher order significant interactions override lower order significant interactions.

Results

Gas exchange

$[CO_2]$ and light had significant ($p<0.05$) interactive effects on net photosynthetic rate (P_n) after 2.5 months (Fig. 4.1a, App. 4). The general trend was that P_n increased with light levels and $[CO_2]$ (Fig. 4.1a). However, the difference in P_n was not statistically significant between 30% and 50% light under ambient $[CO_2]$ or between the two $[CO_2]$ at 30% light (Fig. 4.1a). The stimulation of P_n by the CO_2 elevation was 30, 80 and 34%, respectively, for the 100, 50 and 30% light treatment. After 4.5 months, the above general trend remained and the interaction between $[CO_2]$ and light became statistically insignificant (Fig. 4.1b, App. 5). On average, elevated $[CO_2]$ increased P_n by 68% (Fig. 4.1b). The increase in P_n with increased $[CO_2]$ was 49, 83 and 94%, respectively, for the 100, 50 and 30% light treatments. In general, the stimulation of P_n by CO_2 elevation was greater after 4.5 months than 2.5 months of treatment and the largest increase was at the lowest light treatment (30%).

After 2.5 months, seedlings grown at elevated $[CO_2]$ had significantly ($p<0.05$) lowered the CO_2 compensation point (Γ) (Fig. 4.2a, App.4). There were significant ($p<0.01$) light-species interactions. While light had no effect on the Γ of black spruce, Γ of white spruce was significantly higher at 50% than at 100 and 30% light (Fig. 4.2a). After 4.5 months of treatment, $[CO_2]$ and light had significant ($p<0.05$) interactive effects on the Γ (Fig. 4.2b, App. 5). Seedlings grown at 50 and 30% light at ambient $[CO_2]$ recorded the greatest Γ while no significant differences occurred between other treatment combinations. There was also significant ($p<0.01$) CO_2-species interaction after 4.5 months (Fig. 4.2b, App. 5). Black

spruce grown at elevated [CO_2] had significantly lower Γ while no significant differences occurred among other treatment combinations (Fig. 4.2b).

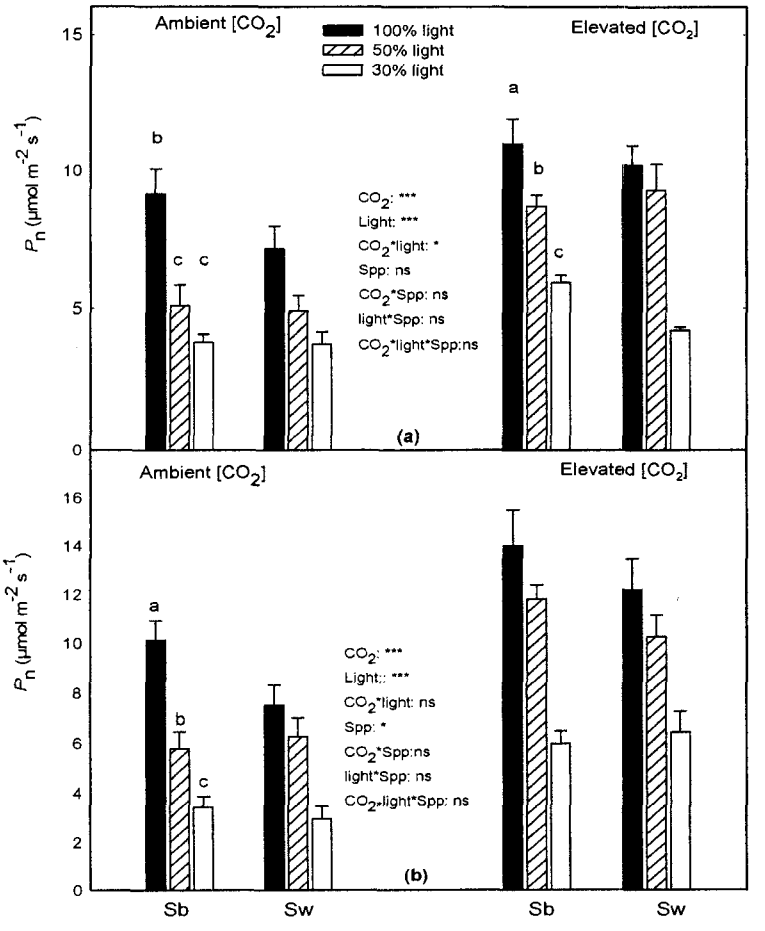

Fig. 4.1: Effects of [CO_2] and light on the net photosynthesis of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In Fig. 4.1a, the letters represent CO_2*light interactions. In Fig. 4.1b, the letters represent light effects. Other explanations are as in Fig. 3.1.

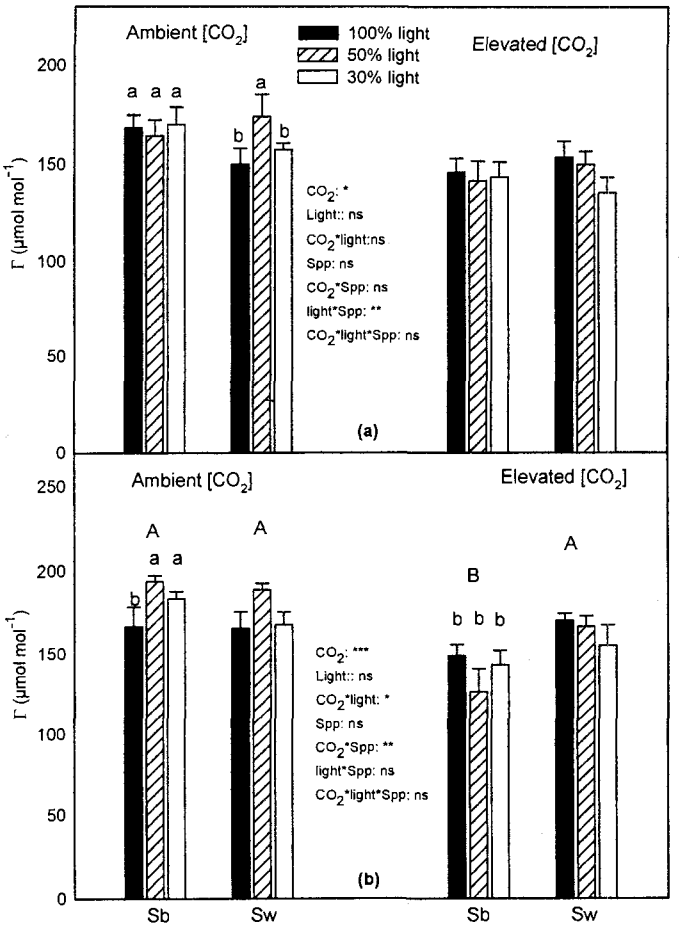

Fig. 4.2: Effects of $[CO_2]$ and light on CO_2 compensation point of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In Fig. 4.2a, the letters represent light*species interactions. In Fig. 4.2b, the lower case letters represent CO_2*light interactions while the upper case letters represent CO_2*species interactions. Other explanations are as in Fig. 3.1.

The CO_2 elevation significantly ($p<0.05$) reduced CE but CE was higher at higher light levels after 2.5 months of treatment (Fig. 4.3a, App. 4). After 4.5 months, all the two way interactions became significant ($p<0.05$), (App. 5). For the $[CO_2]$ - light interaction, at elevated $[CO_2]$, CE decreased with from 100 to 30% light. However at ambient $[CO_2]$,

seedlings at 50 and 30% light had similar responses, with seedlings at full light recording the greatest CE (Fig. 4.3b). For the CO_2 and species interaction, black spruce grown at elevated [CO_2] had significantly lower CE while there was no significant difference among the other treatment combinations (Fig. 4.3b). For the light-species interaction, CE decreased with decreasing light in black spruce while in white spruce seedlings at 50 and 30% light had similar CE (Fig. 4.3b).

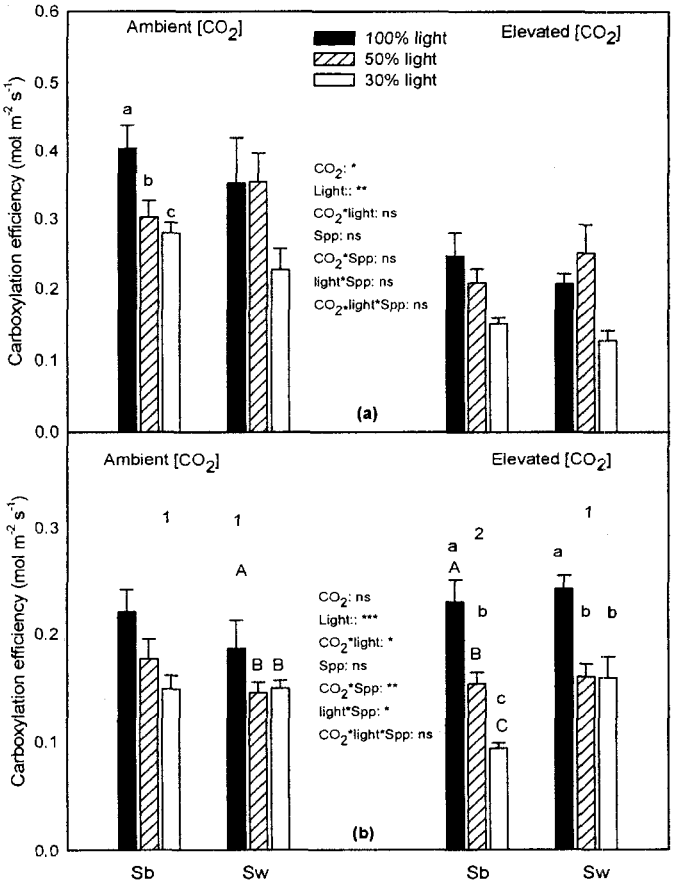

Fig. 4.3: Effects of [CO_2] and light on carboxylation efficiency of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In Fig. 4.3a, the letters represent light effects. In Fig. 4.3b, the lower case letters represent light*species effects while the upper case letters represent CO_2*light effects. The numerics represent CO_2*species effect. Other explanations are as in Fig. 3.1.

The three factors (CO_2, light and species) had significant interactive effects on dark respiration (R_{day}) after 2.5 months (Fig. 4.4a, App. 4) and after 4.5 months of treatment (Fig. 4.4b, App. 5). After 2.5 months, black spruce grown at 100% light at ambient [CO_2] had the greatest R_{day} followed by white spruce grown at 100 and 50% light. There was no significant difference in the R_{day} of white spruce grown at 100 and 50% light at either [CO_2] and in black spruce grown at 50 and 30% light at ambient [CO_2]. Seedlings grown at 30% light under CO_2 elevation recorded the lowest R_{day} among all treatment combinations (Fig. 4.4a).

After 4.5 months, black spruce at 30% light and white spruce at 50% light at elevated [CO_2] had the lowest R_{day} (Fig. 4.4b). Black spruce at 100 and 50% light at ambient [CO_2], white spruce at 100% light at both [CO_2] had the highest R_{day} (Fig. 4b). At ambient [CO_2], R_{day} of black spruce was 6% higher than that of white spruce. However at elevated [CO_2], white spruce's R_{day} was 17% higher than that of black spruce.

CO_2 elevation significantly increased triose phosphate utilisation (TPU) in both measurements (Figs. 4.5a and 4.5b, App. 4 and 5). After 2.5 months, seedlings grown at elevated [CO_2] had a 32% higher TPU and this was increased to 40% after 4.5 months. Light significantly ($p<0.01$) influenced TPU only after 2.5 months (Fig. 4.5a, App. 4). Seedlings grown at 30% had the lowest TPU (Fig. 4.5a) while no significant difference occurred in seedlings at 100 and 50% light. There was no significant interactive effect during both measurement cycles (App. 4 and 5).

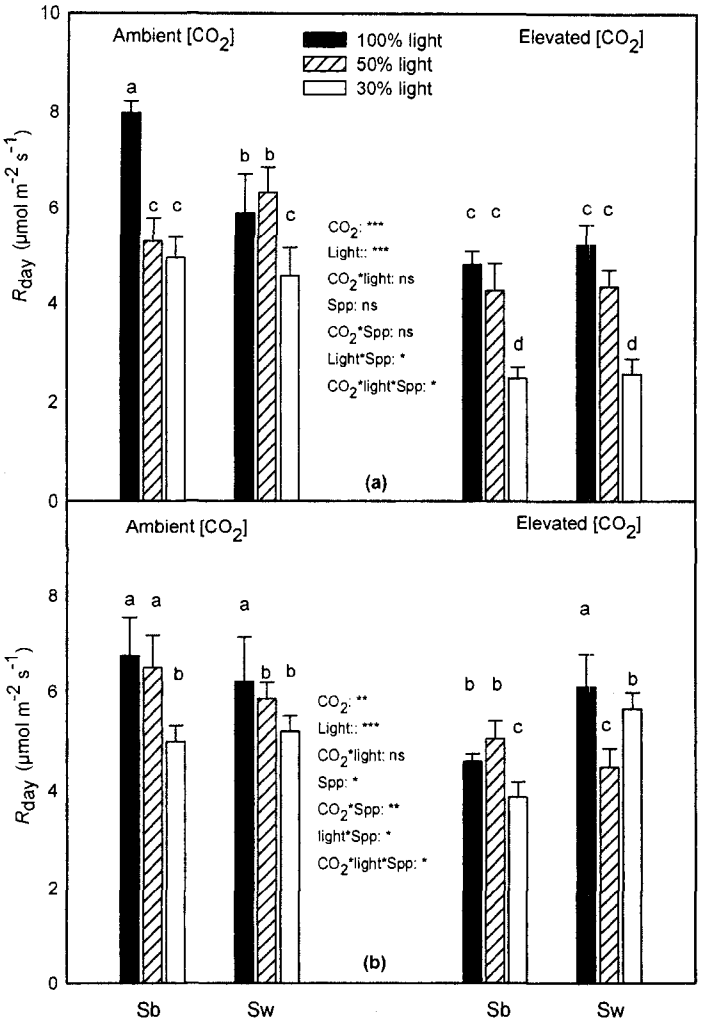

Fig. 4.4: Effects of $[CO_2]$ and light on R_{day} of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In Fig. 4.4a and 4.4b, the letters represent CO_2*light*species interactions. Other explanations are as in Fig. 3.1.

CO_2 elevation significantly reduced stomatal conductance (g_s) by 40% after 2.5 months ($p<0.001$) and 45% after 4.5 months ($p<0.05$) (Figs. 4.6a and 4.6b, App. 4 and 5). Black spruce's g_s was 26% higher ($p<0.05$) than white spruce after 2.5 months (Fig. 4.6a, App. 4) and this became insignificant after 4.5 months (App. 5). There was no significant light effect or significant interactive effects in either measurement (Fig. 4.6b).

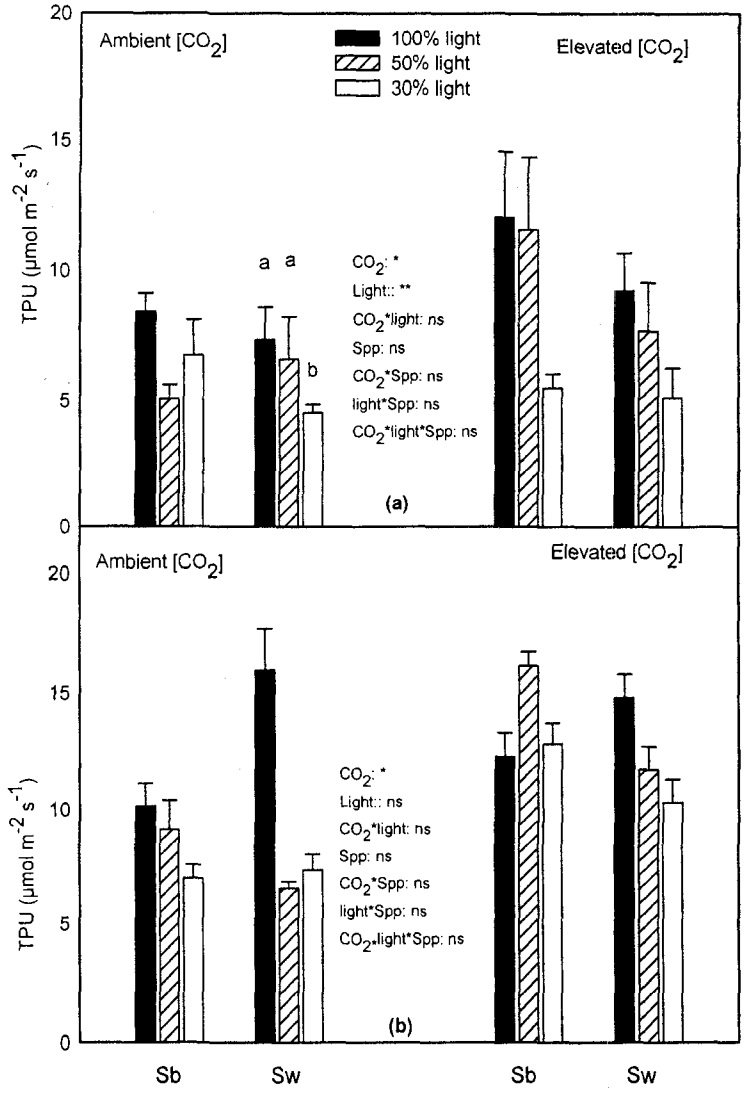

Fig. 4.5: Effects of [CO_2] and light on TPU of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In Fig. 4.5b, the letters represent light effects. Other explanations are as in Fig. 3.1.

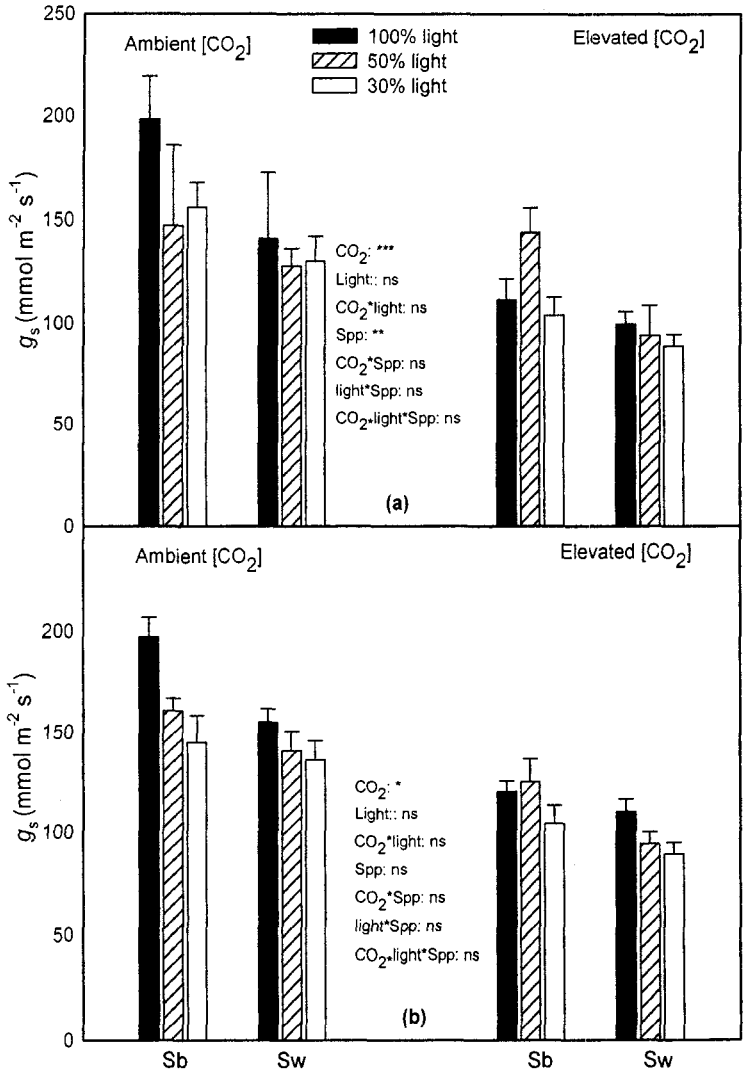

Fig. 4.6: Effects of [CO_2] and light on stomatal conductance of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. Other explanations are as in Fig. 3.1.

CO_2 elevation significantly reduced transpiration rate (E) by 18% after 2.5 months ($p<0.01$) and by 14% after 4.5 months ($p<0.05$) (Figs. 4.7a and 4.7b, App. 4 and 5). Light had significant effects on E after 2.5 ($p<0.001$) and 4.5 months ($p<0.001$) (Figs. 4.7a and 4.7b,

App. 4 and 5). Seedlings grown at 100% light had a 26 and 37% higher *E* over those grown at 50 and 30% light, respectively, after 2.5 months (Fig. 4.7a). After 4.5 months, the *E* of seedlings grown at 100% light was 21 and 31% higher than those grown at 50 and 30% light respectively (Fig. 7b). After 2.5 months, *E* was significantly ($p<0.05$) lower in black spruce than white spruce (Fig. 4.7a, App. 4). After 4.5 months however, the species had similar response to *E* (Fig. 4.7b, App. 5). No significant interactions occurred in either measurement (App. 4 and 5).

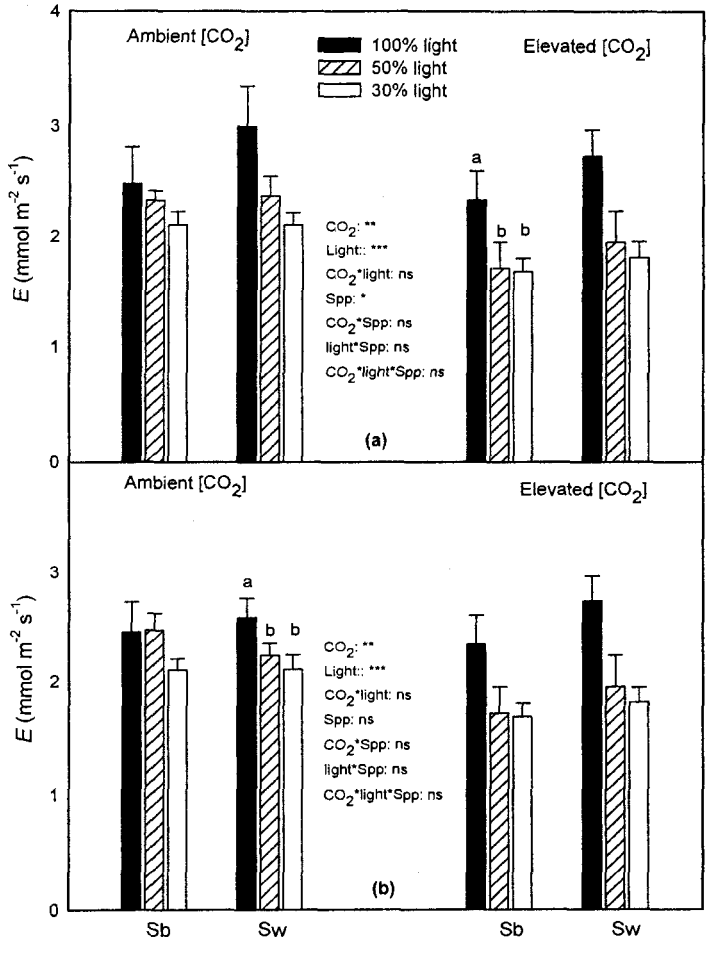

Fig. 4.7: Effects of $[CO_2]$ and light on transpiration rate of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In Fig. 4.7a and 4.7b, the letters represent light effects. Other explanations are as in Fig. 3.1.

[CO_2] and light had significant ($p<0.05$) interactive effects on instantaneous water use efficiency (IWUE) after 2.5 and 4.5 months (Figs. 4.8a and 4.8b, App. 4 and 5). In both measurements, seedlings grown at 100 and 50% light at elevated [CO_2] had the greatest IWUE (Figs. 4.8a and 4.8b), followed by seedlings at 100% light at ambient [CO_2]. Shaded seedlings at ambient [CO_2] and seedlings grown at 30% light in elevated [CO_2] had the lowest IWUE (Figs. 4.8a and 4.8b).

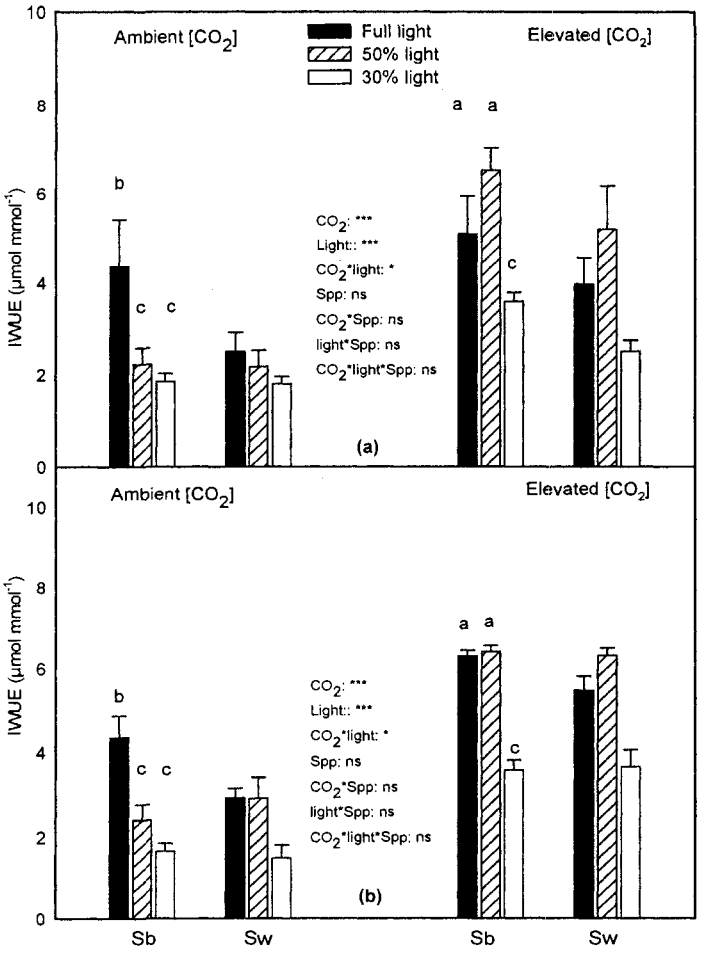

Fig. 4.8: Effects of [CO_2] and light on IWUE of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In Fig. 4.8a and 4.8b, the letters represent CO_2*light interactions. Other explanations are as in Fig. 3.1

Photochemical efficiency of photosystem II (Φ_{PSII})

CO_2, light and species had significant ($p<0.05$) 3-way interactive effects on Φ_{PSII} after 2.5 months (Fig. 4.9a, App. 4). Seedlings of both species grown at elevated [CO_2], white spruce grown at 100 and 30% light at ambient [CO_2] and black spruce grown at 100% at ambient [CO_2] light recorded the highest values (Fig. 4.9a). Black spruce grown at 50 and 30% light and white spruce grown at 50% light under ambient [CO_2] recorded the lowest Φ_{PSII} (Fig. 4.9a). After 4.5 months, neither elevated [CO_2] nor its interaction was significant (Fig. 4.9b, App. 5). However, light and species had significant ($p<0.05$) interactive effects on Φ_{PSII} after 4.5 months (Fig. 4.9b, App. 5) Φ_{PSII} for white spruce decreased with decreasing light while in black spruce, seedlings at 50 and 30% light had a similar response (Fig. 4.9b).

In-vivo carboxylation activity of Rubisco

After 2.5 months of treatment, [CO_2] did not significantly influence Vc_{max} after 2.5 months (Fig. 4.10a, App. 4), but light and species had significant ($p<0.05$) interactive effects on Vc_{max} (Fig. 4.10a, App. 4). The Vc_{max} of black spruce at 100% light was highest among all treatment combinations while it was the lowest for both species under 30% light (Fig. 4.10a). The Vc_{max} values of black spruce at 50% light and white spruce at 100 and 50% light were intermediate and not statistically different from each other (Fig. 4.10a). After 4.5 months, [CO_2] and light had significant ($p<0.01$) interactive effect on Vc_{max} (Fig. 4.10b, App. 5). Seedlings grown at 100% light at elevated [CO_2] had the greatest Vc_{max} (Fig. 4.10b). This was followed by ambient [CO_2]-grown seedlings at 100% light and elevated [CO_2]-grown seedlings at 50% light (Fig. 4.10b). Seedlings grown at 50 and 30% light at ambient [CO_2] and at 30% light under elevated [CO_2] had the lowest Vc_{max} (Fig. 4.10b).

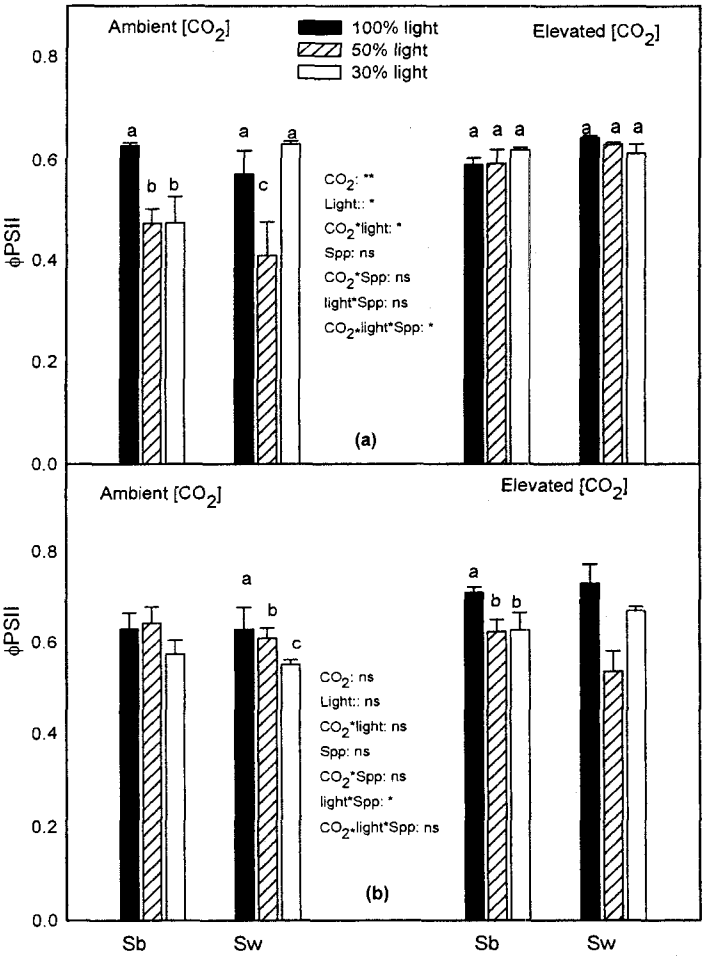

Fig. 4.9: Effects of [CO_2] and light on photochemical efficiency of PSII of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In Fig. 4.9a, the letters represent CO_2*light*species interactions while in Fig. 4.9b they represent light*species interactions. Other explanations are as in Fig. 3.1.

CO_2 elevation had no significant ($p \leq 0.6038$) effect on J_{max} after 2.5 months (Fig. 4.11a, App.5). Light and species, however, had significant ($p<0.001$) interactive effect on J_{max} after 2.5 months (Fig. 4.11a, App.4,). Black spruce grown at 100% light had the highest J_{max} and this was followed by white spruce at 100% light (Fig. 4.11a). No significant difference occurred in the J_{max} between 50% and 30% light or between the two species under shade

(Fig. 4.11a). After 4.5 months, the interaction between species and light became insignificant (App. 5). However, the interaction between [CO_2] and light became significant (App. 5). Seedlings exposed to doubled [CO_2] at full light had the highest J_{max} (Fig. 4.11b). Seedlings at 100 and 50% light at ambient [CO_2] and at 50% light under elevated [CO_2] had a statistically similar J_{max} (Fig. 4.11b). Seedlings at 30% light had the lowest J_{max} at either [CO_2] (Fig. 4.11b).

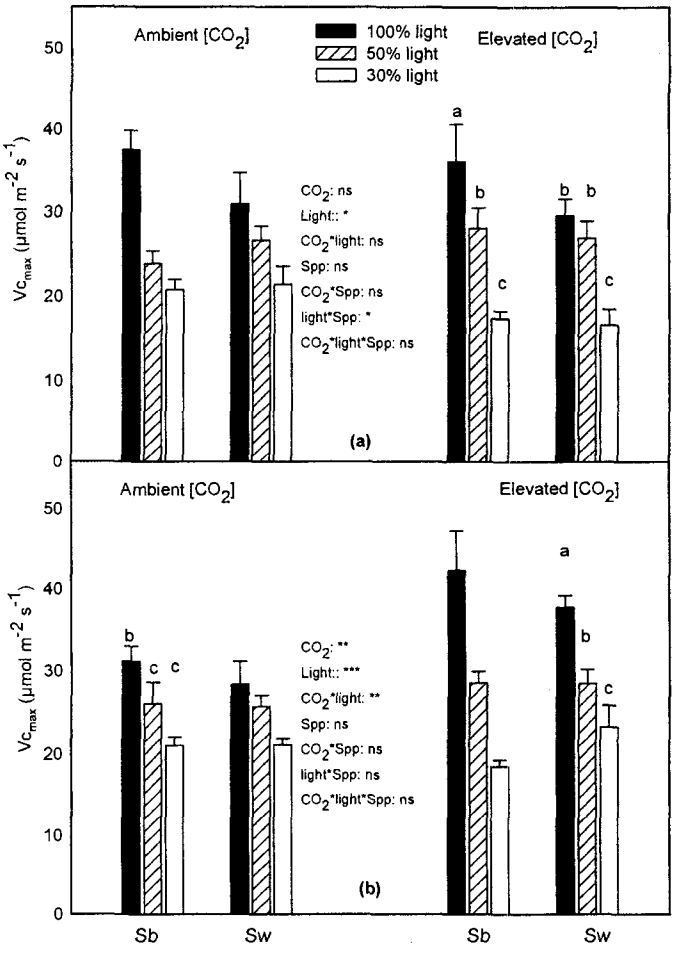

Fig. 4.10: Effects of [CO_2] and light on Vc_{max} of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In Fig. 4.10a, the letters represent light*species interactions and in Fig. 4.10b, they represent CO_2*light interactions. Other explanations are as in Fig. 3.1.

[CO_2], light and species had significant interactive effects on J_t after 2.5 months (Fig. 4.12a, App. 4). All seedlings grown at elevated [CO_2], black spruce at 100% light under ambient [CO_2] and white spruce grown at 30% light under ambient [CO_2] had the highest J_t while black spruce grown at 50% and ambient [CO_2] had the lowest J_t among all treatment combinations (Fig. 4.12a). There were no significant differences in J_t among black spruce at 30% light under ambient [CO_2], white spruce at 50% light and ambient [CO_2], and white spruce at 100% light and ambient [CO_2] (Fig. 4.12a). After 4.5 months, the 3-way interaction became statistically insignificant Fig. 4.12b, App. 5). However, light and species had significant ($p<0.05$) interactive effects on J_t (App. 5). J_t was the highest for white spruce at 100% light and lowest for black spruce at 30% light while there were no significant differences among other treatment combinations.

Light compensation and saturation points

CO_2 elevation significantly ($p<0.001$) reduced LCP after 2.5 months by 44% (Fig. 4.13a, App. 5). Though light was not significant ($p\leq0.0814$) after 2.5 months, light and species had significant ($p<0.05$) interactive effect on LCP (Fig. 4.13a, App. 4). Black spruce's LCP declined with decreasing light (Fig. 4.13a) while that of white spruce did not vary significantly with light conditions and was similar to the value for black spruce at 50% light (Fig. 4.13a). However, the trend for black spruce did not hold under elevated [CO_2] (Fig. 13a). After 4.5 months, the 3-way interaction became significant ($p<0.01$), (App. 5). Black spruce grown at 100% light under ambient [CO_2] had the highest LCP (Fig. 4.13b). With the exception of white spruce at 30% light under ambient [CO_2], seedlings of both species

grown at 30% light recorded the lowest LCP (Fig. 4.13b). At 30% light, LCP was 24 and 33% lower than at 50 and 100% light (Fig. 4.13b).

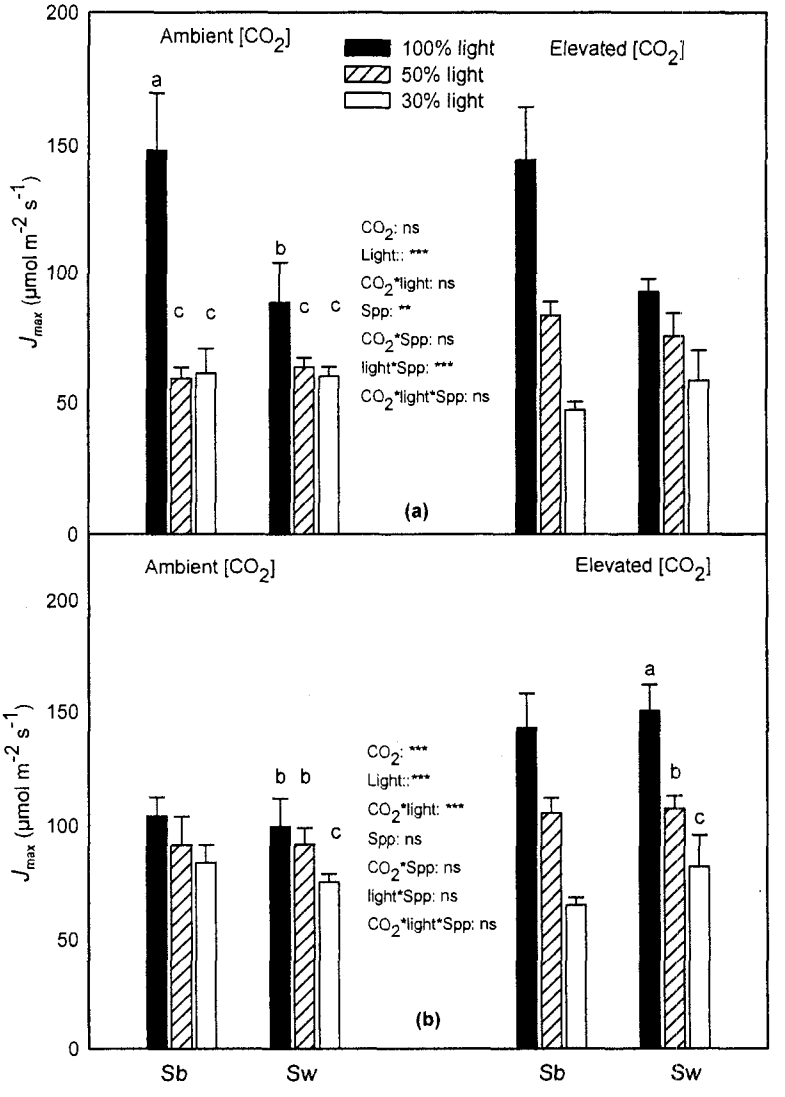

Fig. 4.11: Effects of [CO_2] and light on J_{max} of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In Fig. 4.11a, the letters represent light*species interactions. In Fig. 4.11b, the letters represent CO_2*light interactions. Other explanations are as in Fig. 3.1.

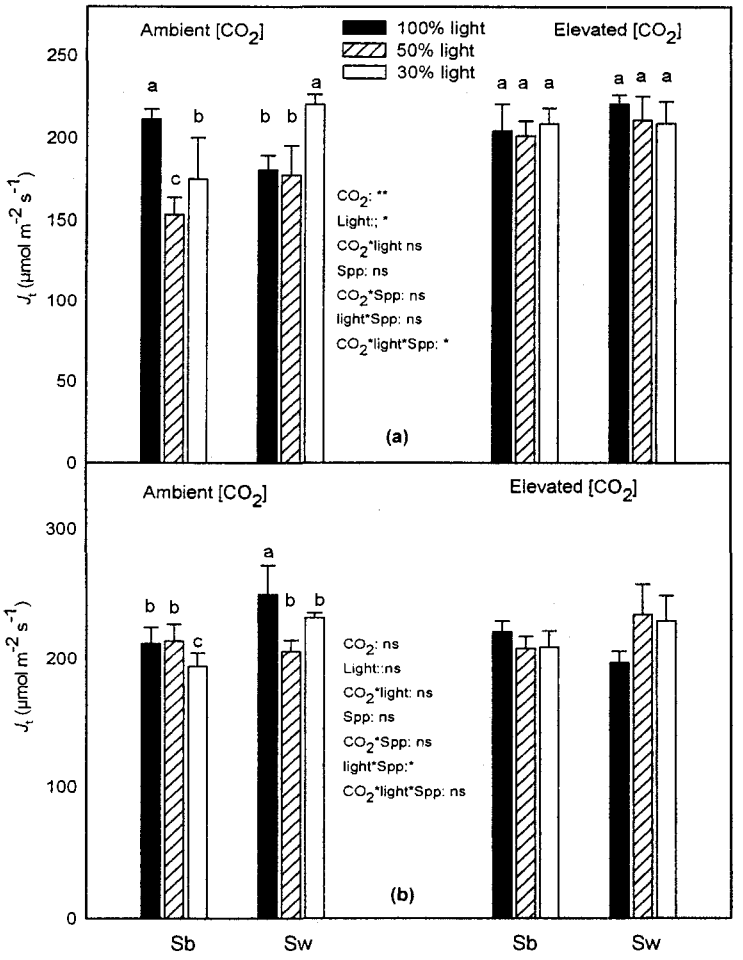

Fig. 4.12: Effects of [CO_2] and light on J_t of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In Fig. 4.12a, the letters represent CO_2*light*species interactions. In Fig. 4.12b, the letters represent light*species interactions. Other explanations are as in Fig. 3.1.

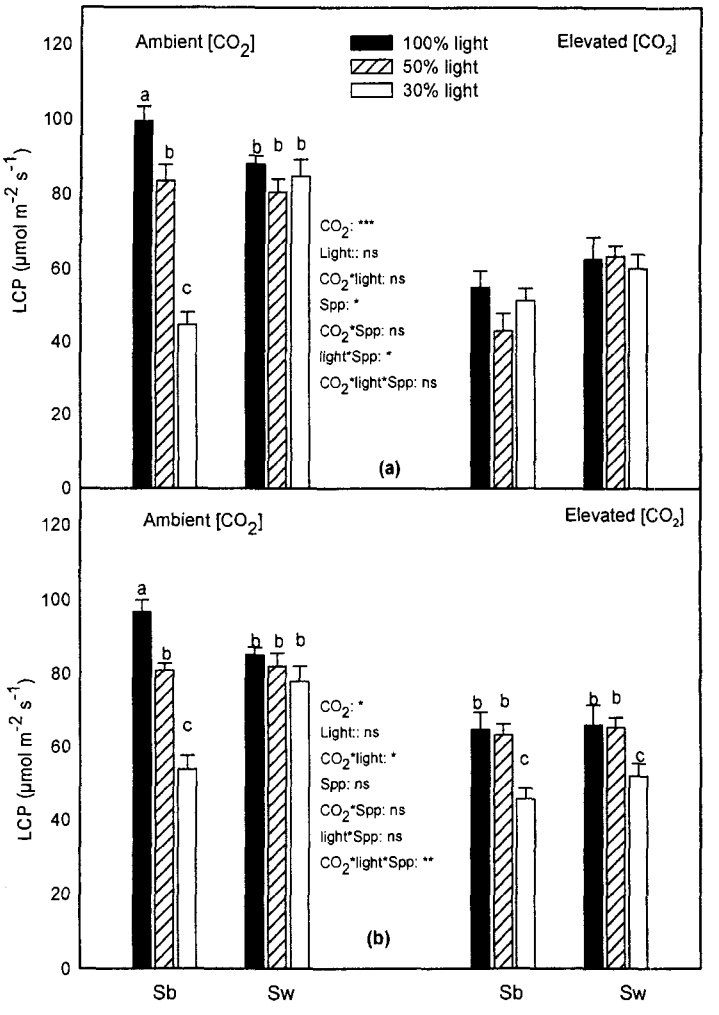

Fig. 4.13: Effects of $[CO_2]$ and light on light compensation point of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In Fig. 4.13a, the letters represent light*species interactions while In Fig. 4.13b, they letters represent CO_2*light*species interactions. Other explanations are as in Fig. 3.1.

$[CO_2]$, light and species had significant ($p<0.05$) interactive effects on LSP after 2.5 and 4.5 months (Figs. 4.14a and 4.14b, App. 4 and 5). After 2.5 months, seedlings of both species at 100% light, white spruce at 50% light and ambient $[CO_2]$ and black spruce at 50% light and elevated $[CO_2]$ had the highest LSP while black spruce grown at ambient $[CO_2]$ and 30%

light had the lowest LSP (Fig. 4.14a). After 4.5 months, all the above trends remained the same with the exception of white spruce grown under ambient [CO_2] where LSP became the highest at 50% light and the difference between 100% and 30% light became statistically insignificant (Fig. 4.14b).

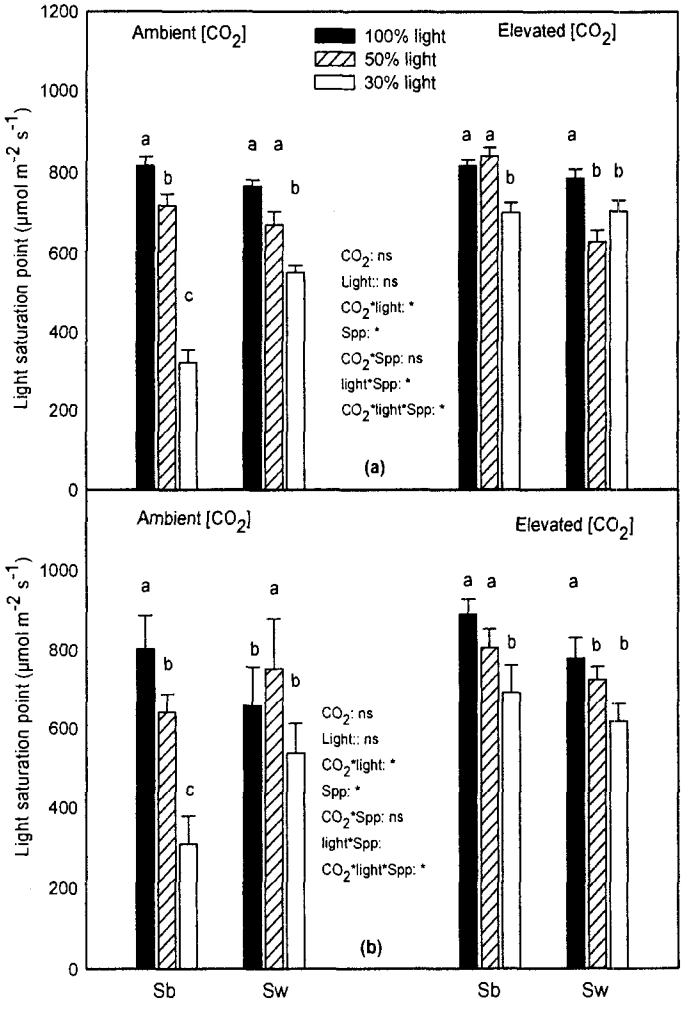

Fig. 4.14: Effects of [CO_2] and light on light saturation point of black spruce (Sb) and white spruce (Sw) seedlings after 2.5 (a) and 4.5 (b) months of treatment. In both Figs. 4.14a and 4.14b, the letters represent CO_2*light*species interactions. Other explanations are as in Fig. 3.1.

Discussion

The absolute amount of photosynthetic stimulation by elevated [CO_2] generally increased with increases in light regime. However, the relative enhancement showed an opposite trend. For example, after 4.5 months, CO_2 elevation enhanced net photosynthesis (P_n) by 49, 83 and 94% at 100, 50 and 30% light, respectively. Thus, the data support our hypothesis that seedlings grown at elevated [CO_2] would have greater rates of photosynthesis and that the relative photosynthetic enhancement by CO_2 elevation would be greater at lower light regimes. The significant interactions between [CO_2] and light at 2.5 months became statistically insignificant after 4.5 months. However, the relative P_n enhancement by elevated [CO_2] after 4.5 months was 3-fold higher at 30% light when compared with the 2.5 month results. While the magnitude of enhancement of P_n at 100 and 50% by CO_2 elevation were almost the same (compared with 2.5-month results), the greater relative increase at 30% light might have accounted for the statistically insignificant CO_2-light interactions after 4.5 months. This is an indication that seedlings grown at low light had a greater light use efficiency under elevated [CO_2], hence the relatively greater P_n increment. These findings also correlate well with our earlier results on growth and biomass (Marfo and Dang 2008). The photosynthetic enhancement reported in this study fagrees with Roberntz and Stockfors (1998), Saxe *et al.* (1998), Ainsworth and Long (2005) and Zhang and Dang (2007). The interactive effects of CO_2 and light on P_n is also in line with those reported earlier by Teskey and Shrestha, (1985) for American beech, white oak, yellow poplar, Northern red oak, sweetgum and loblolly pine. Our findings on the relative enhancement of P_n by elevated [CO_2] at lower light, however, do not support the findings of Herrick and Thomas

(1999), who found that the relative enhancement by elevated [CO_2] in sweetgum increase with increased light.

The relatively more shade tolerant black spruce, was able to take greater advantage of the increased CO_2 by having greater P_n and lower R_{day}. Teskey and Shrestha (1985) found similar results for American beech. This supports our hypothesis that the more shade-tolerant a species is, the more responsive it is to elevated [CO_2] and further supports the findings of both Poorter and Werger (1999) and Marfo and Dang (2008). The greater P_n and fast growing characteristics exhibited by black spruce suggest that irrespective of the light conditions, black spruce be better able to take advantage of the increased atmospheric [CO_2] under future climate than white spruce.

Photosynthetic down-regulation can occur when trees are exposed to elevated [CO_2] over long period of time (Drake *et al.* 1997, Lambers *et al.* 1998, Ainsworth and Long 2005, Körner 2006, Huang *et al.* 2007). According to Lambers *et al.* (1998), a decrease in Rubisco activity is a result of feedback inhibition of photosynthesis due to insufficient sink demand, or reallocation of nitrogen away from the photosynthetic apparatus. Saxe *et al.* (1998) further stress that downregulation of photosynthetic capacity in trees occurs where plants are stressed, especially due to nutrient shortage. Johnsen (1993) reported photosynthetic down-regulation in black spruce exposed to elevated [CO_2] and attributed the observation to the small size of the growing pot. In this study, however, photosynthetic up-regulation occurred. According to Drake *et al.* (1997), a decrease in maximum carboxylation rate of Rubisco (Vc_{max}) over time is a sign of photosynthetic down-regulation. In this study,

however, CO_2 elevation increased Vc_{max} after 4.5 months of treatment (Fig. 4.10b) with a corresponding increase in J_{max} (Fig. 4.11b) and a 16% increase in P_n (Fig. 4.1b) The 54% increase in triose phosphate utilisation (TPU) after the first measurement further supports photosynthetic up-regulation in seedlings exposed to elevated [CO_2]. The increase in TPU further suggests that the sink strength increased, which resulted in the up-regulation of photosynthesis. This result is in agreement with Anderson *et al.* (2001). Furthermore, the CO_2 elevation in this study increased the portioning of electrons to Rubisco carboxylation (J_c) and reduced the portioning of electrons to photorespiration (App. 4 and 5). This finding further strengthens the theory that elevated [CO_2] enhances photosynthesis by promoting Rubisco carboxylation at the expense of photorespiration (Lambers *et al.* 1998, Huang *et al.* 2007). Other studies have reported similar trends photosynthetic up-regulation in *Pinus radiata* (Conroy *et al.* 1988), *Tabebuia rosea* (Ziska *et al.* 1991), *Gossypium hirsutum* L., (Reddy *et al.* 1998), and *Betula papyrifera* (Zhang and Dang 2006).

The reduction in dark respiration rate (R_{day}) by elevated [CO_2] has been reported by several authors (Ainsworth and Long 2005, Zhang and Dang 2005, Körner 2006, Huang *et al.* 2007). CO_2 elevation reduced R_{day} in the two species at all three light levels. However, the reduction was greater at lower light and in black spruce than white spruce. The greater R_{day} at ambient [CO_2] reflects the increase allocation of electrons to photorespiration (J_o) (App. 4 and 5). The greater reduction of R_{day} in shaded seedlings and the significant variation between the two measurement cycles is consistent with literature. The respiratory process of plants depends strongly on the irradiance level and plants grown at low light have reduced respiratory activities (Teskey and Shrestha (1985) and Lambers *et al.* 1998).

Elevated [CO_2] reduces g_s and subsequently transpiration rates (Lodge *et al.* 2001, Huang *et al.* 2007). In this study, CO_2 elevation reduced g_s by 40% after 2.5 months and by 45% after 4.5 months while light had no effect on g_s. Several authors have documented a reduction in g_s in relation to increased [CO_2]. Medlyn *et al.* (2001), for example, reported a 21% reduction in g_s of trees exposed to elevated [CO_2]. As in this study, Morrison (1987) and Lodge *et al.* (2001) observed a 40% reduction in g_s in plants exposed to elevated [CO_2]. However, contrary to these findings, Barton *et al.* (1993), Ellsworth *et al.* (1995), Drake *et al.* (1997) reported that elevated [CO_2] failed to affect g_s. A reduction in g_s in response to elevated [CO_2] generally has greater negative effects on transpiration than photosynthesis (Drake *et al.* 1997), consequently enhancing photosynthetic water use efficiency. This indeed is the case in this study. In this study, the reduction in g_s by elevated [CO_2] resulted in a 15% reduction in E and 80% increase in instantaneous photosynthetic water use efficiency (IWUE). Drake *et al.* (1997) reported similar finding. Tjoelker *et al.* (1998) reported that elevated [CO_2] resulted in decline in mean stomatal conductance of aspen, paper birch, tamarack and black spruce, resulting in 40 – 80% increase in IWUE. Lodge *et al.* (2001) exposed *Quertcus myrtyfolia* Willd. to 350 μmol mol^{-1} above ambient [CO_2] and observed that elevated [CO_2] reduced g_s by 40%, resulting in a 19% decrease in transpiration. Zhang and Dang (2005) also reported similar findings for jack pine and white birch. The increase in IWUE under elevated [CO_2] was due to reduced E and increased in P_n. Higher water use efficiency is a positive adaptation to a limited water supply and plants with greater water use efficiency can perform better in dry environments. Water use efficiency may affect species composition and natural regeneration (D'Alessandro *et al.* 2006) by promoting the reproduction of species that respond to dry site conditions. The data

presented on *P*n, g_s, *E* and IWUE support the hypothesis that an increase in P_n with a reduction in g_s would result in a decrease in *E* and increase in IWUE. While g_s effect on P_n was more of curvilinear nature, *E* had a linear response to g_s. Beadle *et al* (1985) found this trend in mature Scots pines. At lower g_s, vapour pressure difference between the leaf and the air can remain constant while the intercellular [CO_2] declines and this will result in a greater decline in *E* than P_n, leading to an increase in IWUE (Lambers *et al.* 1998).

Light is known to influence g_s through its effect on wavelength sensitive-photoreceptors in the stomatal cells (Lambers *et al.* 1998). However, in this study it had no significant effect on g_s but significantly reduced *E* and IWUE in the shaded seedlings. It therefore appears that the light effect on *E* and IWUE is independent of g_s as reported for pepper plants (Janes 1970). Beadle *et al.* (1985) using mature Scots pine observed that difference in vapour pressure was the cause of changes in g_s while light only had an effect on P_n but not g_s.

Shaded seedlings had lower light compensation point (LCP) and higher light saturation point (LSP) (Figs. 4.13 and 4.14). While CO_2 elevation lowered LCP by 43 and 33% after 2.5 and 4.5 months respectively, LSP was increased by CO_2 elevation (Figs. 4.13 and 4.14). Leakey *et al.* (2002) subjected *Shorea leprosula* Miq. to uniform light and sunflecks and found that elevated [CO_2] lowered LCP by 49 and 33% under uniform light and sunfleck conditions, respectively. Osborne *et al.* (1999) reported that elevated [CO_2] reduced LCP in Indiana strawberry by approximately 42%. Herrick and Thomas (1999) examined the light response of sweetgum leaves exposed to shade and noted a reduced LCP though elevated [CO_2] had no effect. Stewart and Hoddinott (1993) observed in jack pine grown in ultra-violet free light that, CO_2 elevation reduced LCP by 100% and increased LSP by 97%. By

lowering LCP for photosynthesis, elevated [CO_2] allows plants to have greater photosynthetic light use efficiency (Saxe *et al.* 1998) and thus be able to grow in deeper shade (Körner 2006).

The lower light compensation point for species grown under CO_2 elevation implies that under future climatic conditions, the growth rate of regeneration under partial cut harvest regime would have be higher than at present. This is especially important as the lower LCP corresponded with increased P_n and reduced R_{day}. The higher LSP in seedlings exposed to elevated [CO_2] also suggests that plants grown under a forest canopy may be better able to take advantage of periodic high light conditions in the future, as seen during sunflecks and noon hour light.

Chapter 5

GENERAL DISCUSSION AND CONCLUSION

Black spruce and white spruce are two important economic species in the boreal forest, forming the bulk of species cultivated in the region (Bigras and Bertrand 2006). These species occur in a variety of environmental conditions, which are very important in the context of climate change (Hollingsworth *et al.* 2006) because of the large amount of carbon stored in the location of these species (Vogel *et al.* 2005). With increasing atmospheric [CO_2], a good understanding of the response of these two congeneric species to the interactive effects of [CO_2] and light is vital. Such knowledge will provide information for designing appropriate silvicultural techniques.

The morphological and physiological data generally followed a similar trend in this study. This also supports the findings of Armson and Sadreika (1974) and Thompson (1985) that some seedlings` morphological characteristics such as height, generally correlate positively to whole seedling photosynthetic capacity and transpiration efficiency. Seedlings grown at elevated [CO_2] had greater above-and-below-ground biomass in absolute terms with increasing light levels, and a similar trend was observed in the gas exchange net photosynthetic rate. However, the increase was relatively greater at low light levels. In both the morphological and physiological data, black spruce benefitted more from the doubled [CO_2] than white spruce. This is an indication that under future climate conditions when the atmospheric [CO_2] will be doubled, the productivity of black spruce and white spruce will

be greater than at present atmospheric [CO_2] assuming other growth factors are parallel. The relatively more shade tolerant black spruce will be at greater advantage. However, the field environmental conditions are much more complicated and variable than the conditions in the greenhouse and this is a short tem study, therefore, the results of this study should be used with caution.

CO_2 elevation caused a reduced shoot-to-root ratio under the high light regime but an increase under the low light condition. The shift in response to changes in [CO_2] could influence the relative competitiveness of the species under different light conditions in the future climate when the atmospheric [CO_2] is predicted to be substantially higher than at present. This is in general agreement with other studies (Prior *et al.* 1997, Grechi *et al.* 2007).

The growth of trees is related to the light intercepted by the foliage (Saxe *et al.* 1998, Ward and Strain 1999), and this in turn depends on the leaf area and canopy structure as well the height, diameter and number of branches (Huang *et al.* 2007). Taller trees with greater diameter and large number of lateral shoots possess a greater competitive ability (Saxe *et al.* 1998). As such black spruce may be more competitive than white spruce especially under low light conditions in future climates. The greater stem diameter, height and subsequent stem volume in seedlings grown under elevated [CO_2] can enhance the hydraulic conductivity in trees as reported by Saxe *et al* (1998).

The lower transpiration rates coupled with increased water use efficiency of seedlings at elevated [CO_2], especially in black spruce suggest that these trees will be able to grow well under lower moisture regimes in future climates. Though at present atmospheric [CO_2]

white spruce is known to grow mostly on drier sites while black spruce is found on wetter sites (Nienstaedt and Zasada 1990), the greater water use efficiency in black spruce exposed to elevated [CO_2] may enhance the competitive ability of black spruce to grow on drier sites than at present. Seedlings at elevated [CO_2] had a greater Rubisco carboxylation rate and lower rates of oxygenation resulting in lower light compensation points and reduced respiration. Seedlings grown at elevated [CO_2] also had higher light saturation points. These results imply that under increased [CO_2] both species will be able to grow well in a wide range of light regimes with greater photosynthetic efficiency.

With the predicted increase in atmospheric [CO_2], there have been growing concerns from environmental activists about clearcut and even-aged silviculture. This paper's investigations probing the morphological and physiological response of black spruce and white spruce to light may serve to influence the direction of future silvicultural practices. The outcome of this study indicates that the relative photosynthetic and biomass increase under future climates is relatively greater at low light levels. This therefore suggests that un-even aged and partial-cut strategies may become appropriate silvicultural management options at high [CO_2] especially for shade tolerant species. However, a long term research under field conditions is needed for complete understanding of the interactive effects of [CO_2] and light on the productivity of boreal black spruce and white spruce. Additionally, climate changes are complicated. Many other environmental factors will change as well as [CO_2]. This short-term study was conducted under controlled environment conditions. Therefore, the results should be used with caution, particularly when applied in field conditions.

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APPENDICES

Appendix 1: Experiment linear mode and expected mean squares

$$\mathbf{Y_{ijklm} = \mu + C_i + \omega_{(i)j} + \delta_{(ij)} + L_k + CL_{ik} + \omega L_{(i)jk} + \delta_{(ijk)} + S_l + CS_{il} + \omega S_{(i)jl} + LS_{kl} + CLS_{ikl} + \omega LS_{(i)jkl} + \varepsilon_{(ijkl)m}}$$

$$\mathbf{i = 1, 2;\ j = 1, 2;\ k = 1, 2, 3;\ l = 1, 2;\ m^2 = 1, 2, 3...8}$$

Where

Y_{ijklm} = response/measured variable such as height, stem volume etc

μ = the overall mean

C_i = the fixed effect of the i^{th} level of CO_2

$\omega_{(i)j}$ = the whole plot random effect of the j^{th} block (greenhouse) within the i^{th} $[CO_2]$. This is assumed to be identically and independently distributed as N $(0, \delta^2)$

$\delta_{(ij)}$ = the restriction error due to the restriction on CO_2 randomisation

L_k = the fixed effect of the k^{th} level of light

CL_{ik} = the interactive effect of the i^{th} $[CO_2]$ at k^{th} light level

$\omega L_{(i)jk}$ = the interactive effect of the j^{th} whole plot random effect at k^{th} light level

$\delta_{(ijk)}$ = the restriction error on $[CO_2]$ and light

S_l = the fixed effect of the l^{th} species type

CS_{il} = the interactive effect of the i^{th} $[CO_2]$ and l^{th} species type

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[2] For stem volume, biomass, biomass ratios and physiological data, **m** = 3

$\boldsymbol{\omega S_{(i)jl}}$ = the interactive effect of the j^{th} whole plot random effect on l^{th} species type

$\boldsymbol{LS_{kl}}$ = the interactive effect of l^{th} species type at k^{th} light level

$\boldsymbol{CLS_{ikl}}$ = the three-way interactive effect due to the i^{th} $[CO_2]$ at k^{th} light level on l^{th} species type

$\boldsymbol{\omega LS_{(i)jkl}}$ = the three-way interactive effect of the j^{th} whole plot random effect on l^{th} species type at k^{th} light level

$\boldsymbol{\varepsilon_{(ijkl)m}}$ = the error term and is assumed to be identically and independently distributed as $N(0, \delta^2)$

Expected mean square table

	2 F i	2 R j	3 F k	2 F l	8 R m	EMS	Df	Test stat	Ref. Dist
Ci	0	2	3	2	8	$\delta^2 + 48\delta^2\omega + 96\,\Phi(C)$	1	C / ω	1, 2
$\omega_{(i)j}$	1	1	3	2	8	$\delta^2 + 48\delta^2\omega$	2	ω / ε	2, 168
$\delta_{(ij)}$									
L_k	2	2	0	2	8	$\delta^2 + 16\delta^2\omega L + 64\,\Phi(L)$	2	L / ω	2, 4
CL_{ik}	0	2	0	2	8	$\delta^2 + 16\delta^2\omega L + 32\,\Phi(CL)$	2	CL / ω	2, 4
$\omega L_{(i)jk}$	1	1	0	2	8	$\delta^2 + 16\delta^2\omega L$	4	ωL / ε	4, 168
$б_{(ijk)}$									
S_l	2	2	3	0	8	$\delta^2 + 24\delta^2\omega S + 96\,\Phi(S)$	1	S / ω	1, 2
CS_{il}	0	2	3	0	8	$\delta^2 + 24\delta^2\omega S + 48\Phi\,(CS)$	1	CS / ω	1, 2
$\omega S_{(i)jl}$	1	1	3	0	8	$\delta^2 + 24\delta^2\omega S$	2	ωS / ε	2, 168
LS_{kl}	2	2	0	0	8	$\delta^2 + 8\delta^2\omega LS + 32\Phi\,(LS)$	2	LS / ω	2, 4
CLS_{ikl}	0	1	0	0	8	$\delta^2 + 8\delta^2\omega LS + 8\Phi\,(CLS)$	2	CLS / ω	2, 4
$\omega LS_{(i)jkl}$	1	1	0	0	8	$\delta^2 + 8\delta^2\omega LS$	4	ωLS / ε	4, 168
$\varepsilon_{(ijkl)m}$	1	1	1	1	1	δ^2	168	No test[3]	

[3] For all biomass and physiological data, $\varepsilon_{(ijkl)m} = 72$

Appendix 3: Chemical formulation of fertiliser

	Nitrogen (N)	**Phosphorus (P)**	**Potassium (K)**	**Magnesium (Mg)**	**Calcium (Ca)**	**Sulphur (S)**
Target level (mg/l)	150	60	150	40	80	60
Amount in water	-	-	0.5	2.9	15	2
Amount in growth medium			6.2	4.3	3.8	-
Amount to add	150	60	143.3	32.8	31.2	58

Sources of fertiliser	***Amount required per treatment (mg/l)***
Calcium nitrate (19% Ca, 15.5% N)	322.15
Epsom salt (9.8% Mg, 12.9% S)	326.53
Microfine SuperPhosphate (20%P)	597.77
Micromax micronutrient with 12% S	132.50
Muriate of Potash (62% K_2O)	278.44
Ammonium nitrate (NH_4NO_3) 17% NH_4, 17% NO_3	294.12

Appendix 3: ANOVA *p*-values for the effects of [CO_2], light, species (Sp) and their interactions on the height, RCD, stem volume, number of FOLS, SLA, total plant biomass, component biomass, biomass ratios and of black spruce and white spruce seedlings.

Parameter	CO_2	Light	CO_2*Light	Sp	CO_2*Sp	Light*Sp	CO_2*Light*Sp
Height (2.5 months)	0.0001	< 0.0001	0.0012	< 0.0001	0.2739	< 0.0001	0.3227
Height (4.5 months)	<0.0001	< 0.0001	0.7031	< 0.0001	0.139	0.0125	0.0770
RCD (2.5 months)	0.0065	< 0.0001	0.3251	< 0.0001	0.2437	0.7514	0.5969
RCD (4.5 months)	0.0105	0.0292	0.1531	0.4312	0.7532	0.0229	0.183
FOLS (2.5 months)	0.1029	< 0.0001	0.8901	< 0.0001	0.456	0.7795	0.0084
FOLS (4.5 months)	0.0924	< 0.0001	0.8863	< 0.0001	0.478	0.1267	0.5084
Stem volume	< 0.0001	< 0.0001	< 0.0001	0.0119	0.1207	0.0424	0.2395
SLA	0.3330	0.0008	<0.0001	< 0.0028	0.9851	0.4604	0.8451
Total fresh biomass	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Total Dry biomass	< 0.0001	<0.0001	0.057	< 0.0001	0.0005	0.0002	0.0012
Root fresh mass	< 0.0001	< 0.0001	0.0002	0.0514	0.0044	0.2342	0.0058
Root dry mass	< 0.0001	< 0.0001	0.0015	0.0738	0.0022	0.5181	0.014
Shoot fresh mass	< 0.0001	< 0.0001	0.0069	< 0.0001	< 0.0001	< 0.0001	< 0.0001
shoot dry mass	< 0.0001	< 0.0001	0.052	< 0.0001	0.0008	< 0.0001	0.0013
RMR	< 0.0001	< 0.0001	0.0015	0.0738	0.0022	0.5181	0.014
SMR	0.2392	0.0126	0.0034	0.0698	0.1270	0.8600	0.4527
SRR	0.0002	0.0001	< 0.0001	0.0051	0.0157	0.5482	0.4199

Appendix 4: ANOVA *p*-values after 2.5 months for the effects of [CO_2], light, species (Sp) and their interactions on physiology of black spruce and white spruce seedlings grown at 360 and 720 μmol mol^{-1} [CO_2].

Parameter	CO_2	Light	CO_2*Light	Sp	CO_2*Sp	Light*Sp	CO_2*Light*Sp
P_n	<0.0001	<0.0001	0.0347	0.1088	0.8521	0.2650	0.3176
Γ	0.0291	0.3838	0.2532	0.7666	0.3085	0.0098	0.5162
CE	0.0166	0.0018	0.5614	0.1421	0.9058	0.3169	0.9265
R_{day}	0.0001	0.0001	0.5056	0.6144	0.1532	0.0506	0.0203
TPU	0.0138	0.0024	0.1124	0.1142	0.3652	0.8752	0.2653
gs	0.0001	0.3426	0.1760	0.0040	0.6791	0.7933	0.3031
E	0.0036	0.0001	0.5498	0.0505	0.7694	0.3683	0.8517
IWUE	0.0001	0.0008	0.0519	0.0711	0.8370	0.0824	0.4422
PSII	0.0011	0.0223	0.0387	0.4044	0.7480	0.2854	0.0423
Vc_{max}	0.5896	0.0275	0.4067	0.5107	0.1975	0.0286	0.8435
J_{max}	0.6038	0.0001	0.2364	0.0094	0.8633	0.0006	0.7705
J_t	0.0017	0.0350	0.2175	0.1416	0.6698	0.1730	0.0191
J_c	0.0010	0.0001	0.0220	0.6535	0.9407	0.0417	0.0039
J_o	0.4530	0.0223	0.4526	0.2749	0.5459	0.4539	0.1022
LCP	0.0001	0.0814	0.1179	0.0495	0.1802	0.0322	0.1592
LSP	0.0014	0.0008	0.0496	0.6582	0.0871	0.0401	0.0333

Appendix 5: ANOVA *p*-values after 4.5 months for the effects of [CO_2], light, species (Sp) and their interactions on physiology of black spruce and white spruce seedlings grown at 360 and 720 μmol mol^{-1} [CO_2].

Parameter	CO_2	Light	CO_2*Light	Sp	CO_2*Sp	Light*Sp	CO_2*Light*Sp
P_n	0.0001	0.0001	0.2107	0.0541	0.9101	0.8622	0.3484
Γ	0.0001	0.5181	0.0137	0.0993	0.0033	0.2987	0.6547
CE	0.7780	0.0001	0.0395	0.7305	0.0085	0.0532	0.8607
R_{day}	0.0104	0.0014	0.1282	0.0399	0.0076	0.0513	0.0304
TPU	0.0196	0.1177	0.3157	0.9307	0.3893	0.1250	0.9792
gs	0.0480	0.4859	0.4893	0.3902	0.9303	0.5582	0.4346
E	0.0094	0.0001	0.1153	0.2988	0.1894	0.5794	0.8014
IWUE	0.0001	0.0007	0.0427	0.1148	0.3807	0.4341	0.5140
PSII	0.5517	0.0891	0.1328	0.9380	0.2364	0.0174	0.9877
Vc_{max}	0.0010	0.0001	0.0027	0.6834	0.6601	0.1246	0.5362
J_{max}	0.0005	0.0001	0.0003	0.6385	0.6385	0.9561	0.6046
J_t	0.5070	0.4233	0.3314	0.2292	0.2543	0.0096	0.0866
J_c	0.0178	0.0001	0.5108	0.2304	0.8814	0.2524	0.6859
J_o	0.1568	0.0322	0.2725	0.2159	0.2862	0.4907	0.0186
LCP	0.0204	0.7478	0.2200	0.3561	0.9043	0.1216	0.0011
LSP	0.3004	0.1234	0.0347	0.0504	0.2350	0.3826	0.0333